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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 21/06

A1

(11) International Publication Number: WO 92/18641

(43) International Publication Date: 29 October 1992 (29.10.92)

(21) International Application Number: PCT/US92/02977

(22) International Filing Date: 10 April 1992 (10.04.92)

(30) Priority data:

685,101	10 April 1991 (10.04.91)	US
726,606	9 July 1991 (09.07.91)	US
803,842	9 December 1991 (09.12.91)	US

(71) Applicants: THE TRUSTEES OF BOSTON UNIVERSITY [US/US]; Boston University, 80 East Concord Street, A205, Boston, MA 02188 (US). REPLIGEN CORPORATION [US/US]; One Kendall Square, Cambridge, MA 02139 (US).

(72) Inventors: NAVARRO, Javier ; 87 Woburn Street, Andover, MA 01810 (US). THOMAS, Kathleen, M. ; 11 Longwood Avenue, Brookline, MA 02146 (US). WITT, Daniel, P. ; 288 Essex Street, S. Hamilton, MA 02146 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: INTERLEUKIN-8 RECEPTORS AND RELATED MOLECULES AND METHODS

(57) Abstract

Disclosed are cDNAs encoding IL-8 receptors and the recombinant proteins expressed from such cDNAs. The recombinant receptor and receptor fragments and analogs are used in methods of screening candidate compounds for their ability to antagonize interaction between IL-8 and an IL-8 receptor; antagonists are used as therapeutics to reduce inflammation. Antibodies specific for IL-8 receptor (or receptor fragment or analog) and their use as a therapeutic are also disclosed.

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INTERLEUKIN-8 RECEPTORS AND RELATED MOLECULES AND METHODS

Background of the Invention

This invention was made with Government support under #RO1AR39602, #AG00115, and #K04AR01810 awarded by the National Institute of Health. The government has certain rights in the invention.

This invention relates to reducing inflammation.

Under normal circumstances, an orderly progression of host defenses (involving, e.g., T and B lymphocytes, macrophages, and neutrophils) produces a well-controlled immune and inflammatory response that protects the host from offending antigens. However, regulatory dysfunction of any of the systems involved in the host defense can damage host tissue and produce clinically apparent disease. One such dysfunctional condition is inflammation, a pathologic process consisting of a complex set of cytologic and histologic reactions. These reactions occur in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biological agent. Inflammatory disorders include anaphylaxis, systemic necrotizing vasculitis, systemic lupus erythematosus, serum sickness syndromes, psoriasis, and rheumatoid arthritis, and reperfusion injury occurring following periods of ischemia, such as in myocardial infarction or shock. Inflammation may also play a role in homograft rejection.

Neutrophils are cellular components of the blood which play a role in the inflammatory process. When activated (e.g., following infection of the host by a pathogen), neutrophils produce substances that are cytotoxic and amplify the inflammatory response. During intense inflammation, r leas of neutrophil prot olytic

enzymes and oxygen free radicals may cause digestion f cartilage mucopolysaccharide, oxidation of synovial tissue, and widespread damage to the lungs. In addition, chemotactic factors at the site of inflammation induce neutrophil aggregation and adherence to endothelium, causing, e.g., leukostasis in the pulmonary vasculature and cardiopulmonary dysfunction (Jandl, *Blood*, Little, Brown & Co., Boston, 1987).

Interleukin-8 (IL-8) is a 72 amino acid peptide which is produced by a variety of cell types upon activation with interleukin-1 and other stimulatory cytokines (Westwick et al., *Immunology Today* 10:146, 1988). IL-8 has previously been known as neutrophil activating peptide-1 (NAP-1), neutrophil activating factor (NAF), and monocyte-derived neutrophil chemotactic factor (MDNCF). The amino acid sequence of IL-8 has been determined (Lindley et al., *Proc. Natl. Acad. Sci. USA* 85:9199, 1988). IL-8 promotes chemotaxis and degranulation of neutrophils (Djeu et al., *J. Immunol.* 144:2205, 1990). IL-8 has been shown to be a potent chemoattractant for neutrophils *in vitro* and capable of producing a strong inflammatory effect *in vivo* (Colditz et al., *Am. J. Pathol.* 134:755, 1989). In addition, IL-8 has been found to be present in significant quantities in naturally occurring inflammatory conditions such as psoriasis and rheumatoid arthritis. It is likely that IL-8 is a central factor in neutrophil-mediated inflammatory processes. For this reason, inhibitors or antagonists of IL-8 action can be expected to be useful anti-inflammatory agents.

IL-8 action on neutrophils is mediated by a specific receptor (Grob et al., *J. Biol. Chem.* 265:8311, 1990). This glycoprotein has been estimated to be of molecular mass 58,000 Dalt ns and is limited to granulocytic cells, especially neutrophils. This

receptor, which has hitherto not been fully characterized or clon d, can be xpected to be f particular utility in the development of IL-8 inhibitors and antagonists.

Summary of the Invention

In general, the invention features recombinant IL-8 receptor polypeptide. The receptor polypeptide may bind IL-8 with high affinity or with low affinity. Preferably, the receptor includes an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 1 (SEQ ID NO: 1), Fig. 2 (SEQ ID NO: 5), or Fig. 9 (SEQ ID NO: 6). The invention also features a substantially isolated polypeptide which is a fragment or analog of an IL-8 receptor and which includes a domain capable of binding IL-8.

In various preferred embodiments, the receptor is derived from a mammal, preferably, a human or a rabbit.

The invention further features a polypeptide including all or an IL-8-binding portion of an IL-8 receptor transmembrane domain or an IL-8 extracellular domain. Preferably, the polypeptide includes approximately amino acids 1-37 of the amino acid sequence shown in Fig. 1 (SEQ ID NO.:1) or an IL-8-binding fragment thereof; or approximately amino acids 1-50 of the amino acid sequence shown in Fig. 2 (SEQ ID NO.:5) or an IL-8-binding fragment thereof. Preferably, the polypeptide is a recombinant polypeptide or a synthetic polypeptide.

By "IL-8 receptor polypeptide" is meant all or part of a cell surface protein which specifically binds IL-8 and signals the appropriate IL-8-mediated cascade of biological events; it includes receptors which bind IL-8 with either high or low affinity. By a "polypeptide" is meant any chain of amino acids, r gardless of length or post-translational modification (e.g., glycosylation).

By "high affinity" is meant having a K_d which is 10nM or less (and, preferably, having a K_d which is between 0.1 and 10nM). By "low affinity" is meant having a K_d which is greater than 10nM. A "substantially isolated polypeptide" is one which is substantially free of other proteins, carbohydrates and lipids with which it is naturally associated. By a "substantially identical" amino acid sequence is meant an amino acid sequence which differs only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the biological activity of the receptor. Such equivalent receptors can be isolated by extraction from the tissues or cells of any animal which naturally produce such a receptor or which can be induced to do so, using the methods described below, or their equivalent; or can be isolated by chemical synthesis; or can be isolated by standard techniques of recombinant DNA technology, e.g., by isolation of cDNA or genomic DNA encoding such a receptor. By "derived from" is meant encoded by the genome of that organism and present on the surface of a subset of that organism's cells. By "synthetic peptide" is meant one which is produced by chemical, e.g., peptide synthesis.

In another related aspect, the invention features purified DNA which encodes a receptor (or receptor fragment or analog thereof) described above. The purified DNA may encode a high affinity IL-8 receptor or it may encode a low affinity IL-8 receptor. Preferably, the purified DNA is cDNA; is cDNA which encod s a rabbit IL-8 receptor; is cDNA which encodes a human IL-8

receptor; is included in th plasmid F3R; is included in the plasmid 5b1a; is included in the plasmid 4AB.

By "purified DNA" is meant a DNA molecule which encodes an IL-8 receptor (or an appropriate receptor fragment or analog), but which is free of the genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene encoding the IL-8 receptor.

In other related aspects, the invention features vectors which contain such purified DNA and are capable of directing expression of the protein encoded by the DNA in a vector-containing cell; and cells containing such vectors (preferably eukaryotic cells, e.g., mammalian cells, e.g., myeloma cells or hamster lung fibroblast cells). Preferably, such cells are stably transfected with such purified DNA.

The expression vectors or vector-containing cells of the invention can be used in a method of the invention to produce recombinant IL-8 receptor polypeptide and the polypeptides described above. The method involves providing a cell transformed with DNA encoding an IL-8 receptor or a fragment or analog thereof positioned for expression in the cell; culturing the transformed cell under conditions for expressing the DNA; and isolating the recombinant IL-8 receptor protein. By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of genetic engineering, a DNA molecule encoding an IL-8 receptor (or a fragment or analog, thereof). Such a DNA molecule is "positioned for expression" meaning that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of the IL-8 receptor protein, or fragment or analog, ther of).

In yet another aspect, the invention features a purified antibody which binds preferentially to an IL-8 receptor (or a fragment or analog thereof). By "purified antibody" is meant one which is sufficiently free of other proteins, carbohydrates, and lipids with which it is naturally associated to permit therapeutic administration. Such an antibody "preferentially binds" to an IL-8 receptor (or fragment or analog, thereof), i.e., does not substantially recognize and bind to other antigenically-unrelated molecules.

Preferably, the antibody neutralizes the biological activity in vivo of the protein to which it binds. By "biological activity" is meant the ability of the IL-8 receptor to bind IL-8 and signal the appropriate cascade of biological events. By "neutralize" is meant to partially or completely block (e.g., the biological activity of an IL-8 receptor).

In other aspects, the polypeptides or antibodies described above are used as the active ingredient of therapeutic compositions. In such therapeutic compositions, the active ingredient may be formulated with a physiologically-acceptable carrier or anchored within the membrane of a cell. These therapeutic compositions are used in a method of reducing inflammation.

In yet another aspect, the invention features a method of screening candidate compounds for their ability to antagonize interaction between IL-8 and an IL-8 receptor. The method involves: a) mixing a candidate antagonist compound with a first compound which includes a recombinant IL-8 receptor (or IL-8-binding fragment or analog) on the one hand and with a second compound which includes IL-8 n the ther hand; b) determining wh ther the first and second compounds bind; and c) identifying antagonistic compounds as thos which int rfere with th

binding of the first compound to the second compound and/or which reduc the IL-8-m diated release of intracellular Ca^{++}. By an "antagonist" is meant a molecule which inhibits a particular activity, in this case, the ability of IL-8 to interact with an IL-8 receptor and/or to trigger the biological events resulting from such an interaction (e.g., release of intracellular Ca^{++}).

Finally, the invention features chimeric polypeptides, in particular, the chimeric polypeptides include an amino-terminal portion of the sequence shown in Fig. 1 (SEQ ID NO.:1) fused to a carboxy-terminal portion of the sequence shown in Fig. 2 (SEQ ID NO.:5). Preferably, the polypeptide includes approximately amino acids 1-58 of Fig. 1 (SEQ ID NO.:1) or an IL-8-binding fragment thereof fused to approximately amino acids 63-360 of Fig. 2 (SEQ ID NO.:5) and is encoded by F3R/4AB. The invention also features polypeptides which include an amino-terminal portion of the sequence shown in Fig. 2 (SEQ ID NO.:5) fused to a carboxy-terminal portion of the sequence shown in Fig. 1 (SEQ ID NO.:1). Preferably, the polypeptide includes approximately amino acids 1-62 of Fig. 2 (SEQ ID NO.:5) or an IL-8-binding fragment thereof fused to approximately amino acids 59-355 of Fig. 1 (SEQ ID NO.:1) and is encoded by 4AB/F3R. The invention also features DNA encoding such chimeric polypeptides.

The proteins of the invention are involved in the events leading to neutrophil activation and the inflammatory response. Such proteins are therefore useful to treat or, alternatively, to develop therapeutics to treat inflammation. Particular disorders which may be treated using the proteins and/or the methods of the invention include psoriasis, rheumatoid arthritis, vasculitis, as well as reperfusion injury, or any neutr phil-mediat d inflammatory disorder. Preferred

therapeutics include antagonists, e.g., peptide fragments (particularly, fragments derived from the N-terminal extracellular domain), antibodies (particularly, antibodies which recognize and bind the N-terminal extracellular domain), or drugs, which block IL-8 or IL-8 receptor function by interfering with the interleukin: receptor interaction.

Because the receptor component may now be produced by recombinant techniques and because candidate antagonists may be screened in vitro, the instant invention provides a simple and rapid approach to the identification of useful therapeutics. Such an approach was previously difficult for several reasons: (1) because the interaction between IL-8 and its endogenous receptor on the surface of a neutrophil triggers a series of events leading to the release of proteolytic enzymes and oxygen free radicals, and the resultant destruction of the receptor-bearing neutrophil cell; and (2) because of the presence on the surface of neutrophils of related receptors. Isolation of the IL-8 receptor gene (as cDNA) allows its expression in a cell type remote from neutrophils (e.g., J558, SP2 myeloma cells, COS cells, or Chinese hamster lung fibroblast cells), effectively uncoupling the IL-8 receptor from its normal cytotoxic signaling pathway and providing a system for assaying an IL-8:receptor interaction without associated cell death.

Once identified, a peptide- or antibody-based therapeutic may be produced, in large quantity and inexpensively, using recombinant and molecular biological techniques.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments ther of, and from the claims.

Detailed Description

The drawings will first briefly be d scribed.

Drawings

Fig. 1 (SEQ ID NO: 1) shows the nucleic acid sequence and deduced amino acid sequence of a high affinity IL-8 receptor derived from a rabbit source.

Fig. 2 (SEQ ID NO: 5) shows the nucleic acid sequence and deduced amino acid sequence of a low affinity IL-8 receptor derived from a human source.

Fig. 3 is a series of bar graphs which represent the extent of IL-8 binding to four independently-isolated cell lines which inducibly express a high affinity IL-8 receptor.

Fig. 4 is a graph showing IL-8 binding to a low affinity IL-8 receptor as a function of IL-8 concentration.

Fig. 5 is a graph showing MGSA/GROα binding to a low affinity IL-8 receptor as a function of MGSA/GROα concentration and competition by MGSA/GROα with IL-8 for IL-8 receptor binding.

Fig. 6 is a graph showing competition by various ligands for binding to a low affinity IL-8 receptor.

Fig. 7 is a series of bar graphs which represent the extent of IL-8 binding to high affinity/low affinity and low affinity/high affinity chimeric receptors.

Fig. 8 is a graph showing competition by various ligands for binding to a low affinity IL-8 receptor and a high affinity/low affinity chimeric IL-8 receptor.

Fig. 9 (SEQ ID NO: 6) shows the nucleic acid sequence and deduced amino acid sequence of a low affinity IL-8 receptor derived from a rabbit source.

Fig. 10 is a schematic drawing illustrating the structure of the IL-8 receptors.

Fig. 11 is a series of two graphs showing the percentage of total IL-8 binding to a high affinity IL-8 receptor as a function of agonist concentration.

Polypeptides According To The Invention

Polypeptides according to the invention include the entire high affinity IL-8 receptor (as described in Fig. 1, SEQ ID NO: 1) and the entire low affinity IL-8 receptor (as described in Fig. 2, SEQ ID NO: 5 and Fig. 9, SEQ ID NO:6); high affinity receptors bind IL-8 with a K_d of 10nM or less (and, preferably, with a K_d of between 0.1 and 10nM), and low affinity receptors bind IL-8 with a K_d of greater than 10nM. Such receptors may be derived from any source, but are preferably derived from a mammal, e.g., a human or a rabbit. These polypeptides are used, e.g., to screen for antagonists which disrupt an IL-8:receptor interaction (see below). Polypeptides of the invention also include any analog or fragment of the high affinity or low affinity IL-8 receptors capable of interacting with IL-8 (e.g., those derived from the IL-8 receptor N-terminal extracellular domain). Such analogs and fragments may also be used to screen for IL-8 receptor antagonists. In addition, that subset of receptor fragments or analogs which bind IL-8 and are, preferably, soluble (or insoluble and formulated in a lipid vesicle) may be used as antagonists to reduce inflammatory diseases (see below). The efficacy of a receptor analog or fragment is dependent upon its ability to interact with IL-8; such an interaction may be readily assayed using any of a number of standard in vitro binding methods and IL-8 receptor functional assays (e.g., those described below).

Specific receptor analogs of interest include full-length or partial (see below) receptor proteins including an amino acid sequence which differs only by

conservativ amino acid substitutions, for example, substitution f one amino acid f r another f th same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the receptors' ability to bind IL-8 (as assayed below).

Specific receptor fragments of interest include any portions of the IL-8 receptor which are capable of interaction with IL-8, for example, all or part of the N-terminal extracellular domain. Such portions include transmembrane segments 1-7 and portions of the receptor deduced to be extracellular (Fig. 10). Such fragments may be useful as antagonists (as described above), and are also useful as immunogens for producing antibodies which neutralize the activity of the IL-8 receptor in vivo (e.g., by interfering with the interaction between the receptor and IL-8; see below). Extracellular regions may be identified by comparison with related proteins of similar structure (e.g., other members of the G-protein-coupled receptor superfamily); useful regions are those exhibiting homology to the extracellular domains of well-characterized members of the family.

Alternatively, from the primary amino acid sequence, the secondary protein structure and, therefore, the extracellular domain regions may be deduced semi-empirically using a hydrophobicity/hydrophilicity calculation such as the Chou-Fasman method (see, e.g., Chou and Fasman, *Ann. Rev. Biochem.* 47:251, 1978). Hydrophilic domains, particularly ones surrounded by hydrophobic stretches (e.g., transmembrane domains) present themselves as strong candidates for extracellular domains. Finally, extracellular domains may be identified experimentally using standard enzymatic digest analysis, e.g., tryptic digest analysis.

Candidate fragm nts (e.g., all or part of transmembrane segments 2-7 r any extrac llular fragment) are tested for interaction with IL-8 by the assays described herein (e.g., the assay described above). Such fragments are also tested for their ability to antagonize the interaction between IL-8 and its endogenous receptor using the assays described herein. Analogs of useful receptor fragments (as described above) may also be produced and tested for efficacy as screening components or antagonists (using the assays described herein); such analogs are also considered to be useful in the invention.

Of particular interest are receptor fragments encompassing the extracellular amino-terminal domain (or an IL-8-binding fragment thereof); this domain includes approximately amino acids 1-37 of the high affinity IL-8 receptor isolated from a rabbit source, approximately amino acids 1-49 of the low affinity IL-8 receptor isolated from a rabbit source, and approximately amino acids 1-50 of the low affinity IL-8 receptor isolated from a human source. Also of interest are the IL-8 receptor extracellular loops; these include approximately amino acids 94-113, 186-202, and 268-285 of the high affinity IL-8 receptor isolated from rabbits; approximately amino acids 106-118, 183-210, and 272-298 of the low affinity IL-8 receptor isolated from rabbits; and approximately amino acids 107-120, 184-213, and 274-300 of the low affinity IL-8 receptor isolated from humans. Peptide fragments derived from these extracellular loops may also be used as antagonists, particularly if the loops cooperate with the amino-terminal domain to facilitate IL-8 binding. Alternatively, such loops and extracellular N-terminal domain (as well as the full length IL-8 receptor) provid immunogens for producing anti-IL-8 receptor antibodies.

For xample, applicants have produced polyclonal antibodies to loop 2 and loop 3, and to the N-terminal extracellular domain of the high affinity receptor protein isolated from rabbits.

There now follows a description of the cloning and characterization of two IL-8 receptor-encoding cDNAs useful in the invention. These examples are provided for the purpose of illustrating the invention, and should not be construed as limiting.

Cloning and Characterization of a High Affinity and a Low Affinity IL-8 Receptor from a Rabbit Source

The rabbit high affinity IL-8 receptor gene was isolated as follows.

Rabbit peritoneal neutrophils were isolated from rabbits by the method of Zigmond and Tranquillo (-----, 1986) and used as a source of poly(A)$^+$ RNA. The RNA was prepared, transcribed into cDNA, and cDNA fragments inserted into the EcoRI site of λgt11 (all by the methods of Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1989) to produce a rabbit neutrophil cDNA library. 250,000 recombinant plagues were screened for those which hybridized to an antisense oligonucleotide of sequence:

3' TTG ATG AAG GAC GAC TCG GAC CGG ACI CGI CTG GAI TAG TAC 5' (SEQ ID NO: 2)

This probe was designed based on the sequence derived from the second transmembrane domain of G-protein-coupled receptors (see, e.g., Hartig et al., *TIBS* 10:64, 1989).

This probe was 5'-end-labeled with [^{32}P]ATP (Du Pont-New England Nuclear, Boston, MA) and T4 kinase (New England Biolabs, Beverly, MA) by the methods of Maniatis et al., supra. The hybridization conditions were as follows: 6X SSPE, 1% SDS, 0.1% s dium pyrophosphat , 1X D nhardt's, 100 μg/ml poly(A), and 40 μg/ml denatur d

calf thymus DNA at 42°C for 12 h. Filters were washed with 2X SSC, 0.1% SDS at 50°C. After tertiary screening, six plaques were isolated. The insert of one of these plaques, termed F3R was of 2.5 kb in size. This insert was sequenced using Sequenase 2.0 (U.S. Biochemical Corp., Cleveland, OH) according to the method of Sanger et al. *Proc. Natl. Acad. Sci. USA* 74:5469, 1983. It displayed an open reading frame coding for a 354-amino acid protein (M_r = 40,528). The nucleic acid sequence and deduced amino acid sequences are shown in Fig. 1. Putative N-linked glycosylation sites are underlined in the sequence.

Several structural features of the protein deduced from the F3R clone demonstrate that it belongs to the family of G-protein-coupled receptors. First, a hydropathy plot of the deduced protein sequence indicates the existence of seven putative transmembrane segments. Second, the primary structure of F3R shows a high degree of similarity to other G-protein-coupled receptors. In particular, the highest degree of homology is found to G-protein-coupled receptors that bind peptides such as the substance K and P receptors (Masu et al., *Nature* 329:836, 1987;Hershey and Krause, *Science* 247:958, 1990). Third, F3R exhibits several structural features attributed to G-protein-coupled receptors. For example, F3R contains two putative N-linked glycosylation sites in the N-terminus with no signal sequence. It also contains an aspartate at position 80 (i.e., in transmembrane segment II) which is conserved in all G-protein-coupled receptors, and the canonical Asp-Arg-Tyr tripeptide close to the putative transmembrane segment III. Like substance K and P receptors, F3R lacks Asp-113 in the putative transmembrane segment II which appears to be essential for binding of charged amines in adrenergic, muscarinic, dopaminergic, and serotonergic receptors (Dixon et al.,

Cold Spring Harbor Symp. Quant. Biol 53:487, 1988); and lik other G-protein-coupled recept rs, F3R exhibits several critically-located serine and threonine residues which are potential substrates for protein kinases (Benovic et al., *Ann. Rev. Cell Biol.* 4:405, 1988).

To further characterize expression of the F3R gene, the F3R cDNA was employed as a hybridization probe in Northern blot analysis of rabbit neutrophil RNA. RNA was isolated from neutrophils and other tissues by cesium chloride gradient centrifugation (Glisin et al., *Biochemistry* 13:2633, 1974), electrophoresed through 1% agarose formaldehyde gels, and blotted to GeneScreen membranes (Du Pont-New England Nuclear) by the method of Maniatis et al, supra. The blot was probed with a *Bam*HI/*Eco*RI fragment of F3R (652 bases; nucleotides -27 to 625 of the rabbit IL-8 coding sequence) labeled with [^{32}P]dCTP by the random priming protocol of Pharmacia (Piscataway, NJ). The hybridization solution contained 50% formamide, 5X SSPE, 5X Denhardt's, 0.1% sodium pyrophosphate, 1 mg/ml heparin, 100 µg/ml poly(A), 1% SDS, and 200 µg/ml denatured calf thymus DNA. The blot was hybridized at 42°C for 16 h, and then washed with 0.1X SSC and 0.1% SDS at 65°C.

The F3R probe hybridized specifically to a neutrophil RNA molecule of 2.6 kilobases. This confirmed that F3R was expressed in neutrophils and indicated that the F3R clone was nearly full-length. The F3R clone failed to hybridize to RNA isolated from rabbit uterine smooth muscle, skeletal muscle, lung, liver, or brain. It also failed to hybridize to poly(A)$^+$ RNA from fibroblasts, epithelial, and endothelial cells. Promyelocytic HL-60 cells exhibited very low levels of F3R mRNA; differentiated HL-60 cells expressed 20-fold higher l vels of this RNA.

The F3R mRNA was translated in vitro in rabbit reticulocyte lysates by the method of Prom ga Corp. (Madison, WI). A protein of relative mass 30,000-32,000 Daltons was synthesized as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE; carried out by standard techniques; see, e.g., Ausubel et al., *Current Protocols in Molecular Biology*, Green Publishing Associates, New York, 1987). The difference between the calculated M_r of 40,528 and the apparent M_r of about 31,000 was likely due to the fact that membrane proteins frequently exhibit increased mobility relative to soluble protein standards on SDS-PAGE (Bonitz et al., *J. Biol. Chem.* 255:11927, 1980; Rizzolo et al., *Biochemistry* 15:3433, 1979).

Using the methods described above, a cDNA encoding the rabbit low affinity IL-8 receptor was also identified and isolated from the rabbit neutrophil library (described above). This cDNA was subcloned into the EcoRI site of pUC19 to produce plasmid 5b1a. Its nucleic acid sequence was determined by standard techniques and found to be similar, but not identical, to the high affinity receptor clone F3R.

Cloning of a Low Affinity IL-8 Receptor from a Human Source

A human peripheral blood leukocyte λgt11 cDNA library (5' stretch) obtained from Clontech (Palo Alto, CA) was screened with a 652 base pair *Eco*RI/*Bam*HI fragment (including nucleotides -27 to 625) of the rabbit F3R clone. This probe was labeled with [^{32}P]dCTP by random priming as described above. Filters were hybridized with a solution containing 50% formamide, 200 μg/ml denatured calf thymus DNA, 5X SSPE, 1% SDS, 5X Denhardt's solution, and 0.1% sodium pyrophosphate, and incubated at 42°C for 16 hours. Filters were then washed with 0.1X SSC and 0.1% SDS at 65°C. After tertiary

screening, several human clones which hybridiz d to the rabbit IL-8 probe wer isolat d. The insert of one such clone, termed 4AB, was found to be 4.0 kilobases in length; the insert was sequenced on both strands using Sequenase 2.0 (U.S. Biochemical Corp.) according to the method of Sanger et al. (supra). The nucleic acid sequence and deduced amino acid sequence of the human low affinity IL-8 receptor is shown in Fig. 2 (SEQ ID NO: 5).

Alternatively, a human IL-8 receptor-encoding gene may be isolated by hybridization with the full-length F3R probe. This probe is labelled (e.g., radiolabelled) by standard techniques (see, e.g., Ausubel et al., *Current Protocols in Molecular Biology*, supra) and used to probe a human peripheral blood leukocyte library (e.g., the library described above) under low stringency conditions (e.g., hybridization in 50% formamide, 200 μg/ml denatured calf thymus DNA, 5X SSPE, 1% SDS, 5X Denhardt's solution, and 0.1% sodium pyrophosphate at an incubation temperature or 42°C for 16 hours). Filters are washed initially under low stringency conditions (e.g., 2X SSC and 0.1% SDS and an incubation temperature of 50°C) and the stringency progressively increased, through four washes, to a final high stringency wash (e.g., 0.1X SSC and 0.1% SDS and an incubation temperature of 65°C).

The human IL-8 receptor gene may also be isolated by PCR cloning using primer sequences based either on the sequence of clone 4AB, for example:

5' GAATATGGGGAATTTATTATGCAG 3' (SEQ ID NO: 3) and

5' AATGTGACTGTGAAGAGAAGGGAGG 3' (SEQ ID NO: 4);

or based on sequences substantially shared by 4AB, 5b1a, and F3R, for example:

5' GGGAAACTCCCTCGTGATGCTGG 3' (SEQ ID NO: 7) and

5' GTCTGCCAGCAGGACCAGGTTGTAGG 3' (SEQ ID NO: 8).

Primers are synthesized by standard cyan ethyl phosphoramidite chemistry using, e.g., an Applied Biosystems DNA Synthesizer (Foster City, CA).

Human neutrophils are isolated by standard techniques and used as a source of polyA$^+$ RNA as described above. cDNA is synthesized, also as described above, and a neutrophil cDNA library created by insertion of the cDNA fragments into any standard cloning vector, e.g., λgt11. Alternatively, a human peripheral blood leukocyte λgt11 cDNA library (5' stretch) may be purchased from Clontech (Palo Alto, CA).

Approximately 100 ng of human neutrophil or human peripheral lymphocyte cDNA is combined with 1 μg of each of the synthetic primers and polymerase chain reaction is carried out by the directions of the manufacturer (Perkin-Elmer, Norwalk, CT). The resultant PCR product is isolated by electrophoresis and cloned, e.g., into the vector SK+ (Stratagene, LaJolla CA) and amplified in Escherichia coli XL-1 blue (Stratagene).

Polypeptide Expression

Polypeptides according to the invention may be produced by transformation of a suitable host cell with all or part of an IL-8 receptor-encoding cDNA fragment (e.g., the cDNAs described above) in a suitable expression vehicle, and expression of the receptor.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant receptor protein. The precise host cell used is not critical to the invention, however the following host cells are preferred: COS-7, SP-2, NIH 3T3, and Chinese Hamster Ovary cells, Chinese hamster lung fibroblast Dede cells. Such cells are available from a wide range of sources (e.g., the American Type Culture C llection, Rockville, MD). The meth d of transf ction and th choice of

expression vehicle will depend on the host system selected. Mammalian cell transfection methods are described, e.g., in Ausubel et al. (*Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1989); expression vehicles may be chosen from those provided, e.g., in *Cloning Vectors*: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987).

One particularly preferred expression system is the mouse 3T3 fibroblast host cell transfected with a pMAMneo expression vector (Clontech, Palo Alto, CA). pMAMneo provides: an RSV-LTR enhancer linked to a dexamethasone-inducible MMTV-LTR promotor, an SV40 origin of replication which allows replication in mammalian systems, a selectable neomycin gene, and SV40 splicing and polyadenylation sites. DNA encoding the human or rabbit IL-8 receptor or an appropriate receptor fragment or analog (as described above) would be inserted into the pMAMneo vector in an orientation designed to allow expression. The recombinant receptor protein would be isolated as described below. Other preferable host cells which may be used in conjunction with the pMAMneo expression vehicle include COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and CCL 61, respectively).

Another particularly preferred expression system is the COS host cell (ATCC Accession No. CRL 1650) transiently transfected (as described above) with the pSVL vector (Pharmacia) into which an IL-8 receptor-encoding cDNA has been inserted in an orientation which permits expression of the receptor protein.

Alternatively, the high affinity or low affinity IL-8 receptor (or receptor fragment or analog) is produced by a stably-transfected mammalian cell line.

A number of vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (supra); methods for

constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the receptor (or receptor fragment or analog) is cloned into an expression vector which includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the IL-8 receptor-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μM methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

One particularly preferred stable expression system is the myeloma cell line, J558 (ATCC Accession No. TIB6) or SP2 (ATCC Accession No. CRL 1581) stably transfected with pSV2-gpt. pSV2-gpt provides: an SV40 early promotor and a selectable gpt marker (i.e., E. coli xanthine-guanine phosphoribosyl transferase).

Another particularly preferred stable expression system is a Chinese hamster lung fibroblast Dede cell line (ATCC Accession No. CCL39, American Type Culture Collection, Rockville, MD) stably transfected with a pMAMneo vector. This cell line has been used to

inducibly express the rabbit IL-8 r ceptor as foll ws. The F3R receptor cDNA (subcloned into a Bluescript vector, Stratagene, Jolla, CA) was cleaved with XbaI and XhoI, and a fragment of approximately 1700 bp was isolated and inserted into an NheI/XhoI-digested pMAMneo expression vector (Clontech, Palo Alto, CA), to create F3R-pMAMneo. F3R-pMAMneo directs the expression of the rabbit high affinity IL-8 receptor protein under the control of the glucocorticoid-inducible mouse mammary tumor virus promoter. F3R-pMAMneo was used to transfect Chinese hamster lung fibroblast Dede cells (ATCC No. CCL39, American Type Culture Collection, Rockville, MD) using the Lipofectin procedure of BRL (Gathersburg, MD). Transfected cells were selected by growth in medium which included 500μg/ml Geneticin (Sigma Chemical Co., St. Louis, MO). Four G418-resistant clones, termed H1, H9, H11, and H12, were isolated by standard techniques. IL-8 receptor protein was produced in such cells following a 24 hour treatment with 1μM dexamethasone. The ability of the receptor-expressing cells to bind IL-8 was assayed (as described below for Tables 1 and 2), and the results are shown in Fig. 3. This system may be used to inducibly express any polypeptide of the invention.

Alternatively, transfection of the Chinese hamster lung fibroblast Dede cell line (CCL39) with vector RC/CMV (Invitrogen, San Diego, CA) using the methods described above provides a preferred system for the constitutive expression of the polypeptides of the invention.

Expression of the recombinant receptor (e.g., produced by any of the expression systems described herein) may be assayed by immunological procedures, such as Western blot or immunoprecipitation analysis of recombinant cell extracts, or by immunofluorescence of intact rec mbinant cells (using, e.g, the methods described in Ausubel et al., supra). Recombinant

receptor protein is detected using an antibody directed to the receptor. One such antibody is described below; also described below are methods for producing other IL-8 receptor antibodies using, as an immunogen, the intact receptor or a peptide which includes a suitable IL-8 receptor epitope. To detect expression of an IL-8 receptor fragment or analog, the antibody is preferably produced using, as an immunogen, an epitope included in the fragment or analog.

Once the recombinant IL-8 receptor protein (or fragment or analog, thereof) is expressed, it is isolated, e.g., using immunoaffinity chromatography. In one example, IL-8 or an anti-IL-8 receptor antibody (e.g., the IL-8 receptor antibody described below) may be attached to a column and used to isolate intact receptor or receptor fragments or analogs. Lysis and fractionation of receptor-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra). Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

Receptors of the invention, particularly short receptor fragments, can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL).

Assays for IL-8 Receptor Binding and Function

Useful receptor fragments or analogs in the invention are those which interact with IL-8. Such an interaction may be detected by an in vitro binding assay (see below). The receptor component may also be assayed functionally, i.e., for its ability to bind IL-8 and

mobilize Ca^{++} (see below). These assays include, as components, IL-8 and a r combinant IL-8 receptor (or a suitable fragment or analog) configured to permit detection of binding.

IL-8 may be obtained from Genzyme (Cambridge, MA).

Preferably, the IL-8 receptor component is produced by a cell that naturally presents substantially no receptor, e.g., by engineering such a cell to contain nucleic acid encoding the receptor component in an appropriate expression system. Suitable cells are, e.g., those discussed above with respect to the production of recombinant receptor, such as the myeloma cells, J558 or SP2.

In vitro assays to determine the extent of IL-8 binding to the IL-8 receptor may be carried out using either whole cells or membrane fractions. A whole cell assay is preferably performed by fixing the cell expressing the IL-8 receptor component to a solid substrate (e.g., a test tube, a microtiter well, or a column) by means well known to those in the art (see, e.g., Ausubel et al., supra), and presenting labelled IL-8 (e.g., ^{125}I-labelled IL-8). Binding is assayed by the detection label in association with the receptor component (and, therefore, in association with the solid substrate).

The assay format may be any of a number of suitable formats for detecting specific binding, such as a radioimmunoassay format (see, e.g., Ausubel et al., supra). Preferably, cells transiently or stably transfected with an IL-8 receptor expression vector (see above) are immobilized on a solid substrate (e.g., the well of a microtiter plate) and reacted with IL-8 which is detectably labelled, e.g., with a radiolabel or an nzyme which can be assayed, e.g., alkaline phosphatase or hors radish p roxidase.

In a typical experiment using isolated membranes, COS cells were transiently transfected with varying amounts of the rabbit IL-8 receptor-expressing clone F3R-pSVL (see above). Membranes were harvested by standard techniques and used in an in vitro binding assay (see below). ^{125}I-labelled IL-8 was bound to the membranes and assayed for specific activity; specific binding was determined by comparison with binding assays performed in the presence of excess unlabelled IL-8. The results are shown in Table 1.

TABLE 1

Transfected DNA (μg)	Non-Specific Binding (cpm)	Specific Binding (cpm)
0	470	383
1	602	3837
2	589	6594
3	541	8620
4	601	8137

In another typical experiment using whole cells, COS cells were transiently transfected with 8 μg of the human IL-8-expressing clone 4AB-pSVL (see above). Cells were harvested after three days and 2.5 nM ^{125}I-labelled IL-8 was added to approximately 1 X 10^5 whole cells (in 200 μl PBS). Cells were incubated with IL-8 for 45 minutes at 4°C, pelleted by centrifugation, rinsed with cold phosphate buffered saline, and the cell-bound radioactivity measured in a gamma counter. Specific binding was determined by comparison with binding assays performed in the presence of excess (i.e., 250 nM) unlabelled IL-8.

The results are shown in Table 2.

TABLE 2

Transfected DNA (8 μg)	Non-Specific Binding (cpm)	Specific Binding (cpm)
pSVL	385	0
F3R-pSVL	904	3663
4AB-pSVL	471	2521
4AB-pSVL	715	2393

Alternatively, IL-8 may be adhered to the solid substrate (e.g., to a microtiter plate using methods similar to those for adhering antigens for an ELISA assay; Ausubel et al., supra) and the ability of labelled IL-8 receptor-expressing cells to bind IL-8 (e.g., labelled with ^{3}H-thymidine; Ausubel et al., supra) can be used to detect specific receptor binding to the immobilized IL-8.

In one particular example, a vector expressing the IL-8 receptor (or receptor fragment or analog) is transfected into myeloma cells (e.g., J558 or SP2 cells) by the DEAE dextran-chloroquine method (Ausubel et al., supra). Expression of the receptor protein confers binding of detectably-labelled IL-8 to the cells. IL-8 does not bind significantly to untransfected host cells or cells bearing the parent vector alone; these cells are used as a "control" against which the binding assays are measured. Tissue culture dishes (e.g., 10 cm. dishes) are seeded with IL-8 receptor-expressing myeloma cells (approximately 750,000 cells, dish) 12-18h post-transfection. Forty-eight hours later, triplicate dishes are incubated with 0.5nM radioiodinated IL-8 (200 Ci/mmol) and binding to the receptor-bearing cells is assayed (e.g., by harvesting the cells and assaying the amount of detectable label in association with the cells). Cells which specifically bind labelled IL-8 are thos which exhibit a level of binding (i.e., an amount

of detectable label) which is greater than that of the control cells.

Alternatively, IL-8 receptor encoding RNA (prepared as described below) is injected into Xenopus laevis oocytes by standard methods. The RNA is translated in vivo in the oocytes, and the IL-8 receptor protein is inserted into the cell membrane. To test for IL-8 binding, oocyte membranes are prepared by sucrose gradient centrifugation (by the method of Colman, *Transcription and Translation*, IRL Press, Oxford, 1986) and ^{125}I-labelled IL-8 is added, and the membrane preparation subjected to vacuum filtration through Whatman GF/C filters (by the method of Williamson, *Biochemistry*, 27:5371, 1988).

A recombinant receptor may also be assayed functionally for its ability to mediate IL-8-dependent mobilization of calcium. Cells, preferably myeloma cells, transfected with an IL-8 expression vector (as described above) are loaded with FURA-2 or INDO-1 by standard techniques. Mobilization of calcium induced by IL-8 is measured by fluorescence spectroscopy as previously described (Grynkiewicz et al., *J. Biol. Chem.* 260:3440, 1985).

Characterization of Ligand Binding to Recombinant IL-8 Receptors: Affinity of IL-8 Receptors for the IL-8 Ligand

The K_d of the high affinity F3R receptor was determined as follows. pSVL-F3R-transfected COS-7 cell membranes (at a constant amount) were incubated in phosphate buffered saline containing either ^{125}I-labelled IL-8 at a concentration of between 0 and 50nM or were incubated in phosphate buffered saline containing 0.3nM ^{125}I-labelled IL-8 and increasing amounts of unlabelled IL-8; incubation was for 45 minutes at room temperature. The binding reacti n was terminated by addition of 10 ml ice-c ld PBS supplem nted with 1mg/ml BSA, and the

r action mixtur was subjected to vacuum filtration through a Whatman GF/C filter which had been presoaked in 0.3% polyethyleneimine and a subsequent washing with 10 ml PBS containing 1mg/ml BSA. The amount of radioactivity retained on the filter was determined. Using such a membrane binding assay, the F3R receptor K_d was calculated to be 1.4nM (Fig. 11).

IL-8 binding to the low affinity IL-8 receptor (4AB) was measured as follows. 5 X 10^6 COS cells were transiently transfected with 8 μg of the human IL-8-expressing clone 4AB-pSVL (see above). After 3 days, cells were rinsed twice with 7 ml phosphate buffered saline (PBS) and once with 7 ml PBS/1mM EDTA, and incubated in 7 ml of PBS/1mM EDTA at 37°C for 5-10 minutes. The cells were then collected, added to 25 ml of ice cold PBS/0.1% bovine serum albumin (BSA), counted, pelleted by centrifugation, and resuspended in ice cold PBS/0.1% BSA at a concentration of 2 X 10^7 cells/ml. To test IL-8 binding, ^{125}I-labelled IL-8 (at a concentration of between 0 and 20nM) was added to 0.6-1 X 10^6 whole cells (in 100μl PBS/0.1% BSA), incubation was allowed to proceed for 60 minutes at 0°C, and cells were filtered through GF/C filters soaked with 0.3% polyethylenimine (PEI; Sigma, St. Louis, MO), rinsed with cold PBS, and the cell-bound radioactivity measured in a gamma counter. Specific binding was determined by comparison with binding assays performed in the presence of a 300-fold excess of unlabelled IL-8.

The averaged results of three such experiments are shown in Fig. 4. The insert of Fig. 4 depicts a Scatchard transformation of the graphical binding data. The K_d for the low affinity receptor was calculated to be approximately 31nM; this may be compared with the K_d of 1.4nM measur d for the high affinity IL-8 receptor F3R (supra).

IL-8 binding to the 4AB receptor was subsequ ntly measured by the following assay using stably transf cted cells, and the Kd was calculated to be approximately 8.4 nM. The 4AB coding region was subcloned into the HindIII/XbaI sites of the plasmid RC/CMV (Invitrogen) to create pRC.4AB. CHODG44 cells, a double DHFR mutant cell line (gift of Lawrence Chaisen, Columbia University, New York, NY), were stably transfected with pRC.4AB expression vector and a subcloned expressing line was isolated (4ABCH033). Two to three days after passage, the cells were rinsed twice with PBS and treated as described above. To test IL-8 binding, ^{125}I-labelled IL-8 (1.0 to 2.0 nM) was added to samples containing 2.5 x 10^5 cells and increasing amounts of unlabelled IL-8. Incubation was allowed to proceed for 60 min. at 0°C. The cells were filtered through GF/C filters and cell-bound radioactivity was measured as described.

Specific binding was determined by comparison with binding assays performed in the presence of a 500-fold excess of unlabelled IL-8. Binding data was analyzed by non-linear least-squares curve fitting, using the generalized model for complex ligand-receptor systems (Hoffman et al., 1979, Life Sci., 24:1739) and EBDA/LIGAND programs (McPherson, 1985, Kinetic, EBDA, Ligand, Lowry; A collection of radioligand binding analysis programs, Cambridge, U.K.; Biosoft). The results demonstrate saturable, specific binding of [^{125}I] IL-8, and Scatchard analysis of the binding data reveal a single binding site with a Kd of 8.4 nM.

<u>Characterization of Ligand Binding to Recombinant IL-8 Receptors: Specificity of IL-8 Receptors for Related Ligands</u>

The high and low affinity IL-8 receptors were also tested for their ability to bind related ligands.

Experiments wer carried out as described abov ; ^{125}I-labelled MGSA/GROα (Moser et al., *J. Exp. Med.* 171:1797, 1990; Richmond et al., *EMBO J.* 7:2025, 1988; and Anisowicz et al., *Proc. Natl. Acad. Sci. USA* 84:7188, 1987) was added at concentration of between 2 and 7.5nM. Nonspecific binding was determined by adding a 300-fold excess of unlabelled MGSA/GROα or unlabelled IL-8. As shown in Fig. 5, the low affinity IL-8 receptor encoded by 4AB bound the ligand MGSA/GROα and is displaced similarly with either unlabelled MGSA/GROα or unlabelled IL-8. In contrast, no binding of MGSA/GROα was detectable to the high affinity F3R receptor protein (not shown).

Competition experiments were carried out as follows. COS cells were transiently transfected with 4AB-pSVL (as described above). After 3 days, cells were harvested as described above and resuspended in ice cold PBS/0.1% BSA at a concentration of 1.38×10^7 cells/ml. To test ligand binding, ^{125}I-labelled IL-8 (at a concentration of 5nM) was added to a mixture of 6.9×10^5 whole cells expressing the low affinity receptor (in 100µl PBS/0.1% BSA) and unlabelled ligand (specifically, IL-8 at a concentration of between 0 and 5000nM, PF4 at a concentration of between 50 and 5000nM, MGSA/GROα at a concentration of 50 or 500nM, or FMLP at a concentration of between 50 and 5000nM). Cells were incubated in the presence of ligand for one hour at 4°C, filtered through GF/C filters which had been soaked in 0.3% PEI, rinsed with cold PBS/0.1% BSA, and the cell-bound radioactivity measured in a gamma counter.

As shown in Fig. 6, IL-8 and MGSA/GROα successfully competed with IL-8 for binding to the low affinity receptor. Two other peptide ligands, PF4 and FMLP (Deuel et al., *Proc. Natl. Acad. Sci. USA* 78:4585, 1981; Coats and Navarro, *J. Biol. Chem.* 265:5964, 1990)

had little r no effect on IL-8 binding. Thus, th low affinity receptor was not absolutely specific for IL-8; rather, it bound other closely-related members of the IL-8 family. In contrast, the high affinity receptor was specific for IL-8 among the ligands measured. Additional competition experiments were carried out to further characterize the ability of IL-8 receptors F3R and 4AB, and chimeric receptors F3R/4AB and 4AB/F3R, to bind related ligands. COS cells were transiently transfected with vectors expressing each of the receptors. Cells were harvested as described above and resuspended in ice cold PBS/0.1% BSA. To test ligand binding, 2.5×10^5 cells were added to 2 nM [^{125}I] IL-8 in the presence of the unlabeled ligands PF4, MGSA/GROa, NAP-2 and fMLP.

The mixtures were incubated for 60-90 minutes on ice, then terminated by addition of 10 ml ice cold PBS/0.1% BSA followed by vacuum filtration through GF/C filters as described above. Binding data were analyzed by non-linear least-squares curve fitting using the methods of Hoffman et al. (*supra*) and McPherson (*supra*).

The experiments indicated that MGSA/GRO and NAP-2 can compete for binding to the 4AB receptor, but show only very weak binding to the F3R receptor, suggesting that the 4AB receptor is more promiscuous than the F3R receptor. The chimeric receptor containing the F3R extracellular N-terminal domain fused to the backbone of 4AB exhibits a ligand binding profile approximately the F3R subtype, whereas a receptor chimera containing the 4AB extracellular domain fused to the F3R backbone shows a ligand binding profile resembling the human 4AB receptor subtype. These results are consistent with the theory that the N-terminus of the IL-8 receptor is a major determinant of the IL-8 receptor subtype specificity.

Construction of High Affinity/Low Affinity Chimeric IL-8 Receptors

To construct complementary high affinity/low affinity chimeric receptors, the expression vectors F3R-pSVL and 4AB-pSVL (described below) were each digested with XhoI and CelII, and a fragment encoding the amino terminus of one receptor was exchanged for a fragment encoding the amino terminus of the other receptor. Specifically, a 271 bp XhoI-CelII fragment of F3R containing the first 58 codons (i.e., up to and including Ser 58 of Fig. 1) was excised from F3R-pSVL and cloned into a XhoI-CelII ended 4AB-pSVL backbone. In a separate construction, a 283 bp XhoI-CelII fragment of 4AB containing the first 62 codons (i.e., up to and including Ser 62 of Fig. 2) was likewise excised from 4AB-pSVL and cloned into a XhoI-CelII ended F3R-pSVL backbone. Two chimeric IL-8 receptor genes were thus created; one encoding the amino-terminal 58 amino acids of rabbit F3R fused to the 298 carboxy-terminal amino acids of human 4AB (termed F3R/4AB) and the second encoding the amino-terminal 62 amino acids of human 4AB fused to the 297 carboxy-terminal amino acids of rabbit F3R (termed 4AB/F3R).

Mapping of the IL-8 Receptor Binding Domain

Using IL-8 binding assays (e.g., those described above), the affinity of IL-8 for the rabbit F3R receptor has been found to be greater than its affinity for the human 4AB receptor (specifically, K_d = 1.4nM and K_d = 31nM, respectively). This difference in affinity was used to identify the IL-8 binding domain as follows.

COS cells were transiently transfected with F3R/4AB-pSVL or 4AB/F3R-pSVL chimeric receptor expression plasmids (described above), and cells were harvested and washed as described above. To 4-5 x 10^6 transfect d cells (in 100μl PBS/0.1% BSA) was added 1, 5, r 10nM

^{125}I-labelled IL-8. Cells were then incubated in the presence of labelled and unlabelled ligand for one hour at 4°C, filtered through GF/C filters which had been soaked in 0.3% PEI, rinsed with cold PBS/0.1% BSA, and the cell-bound radioactivity measured in a gamma counter. Specific binding was determined by comparison with binding assays performed in the presence of excess (i.e., 0.3-3nM) unlabelled IL-8.

As shown in Fig. 7, IL-8 bound F3R/4AB more readily than it bound 4AB/F3R. The amount of IL-8 binding the chimeric proteins mirrored the amount of IL-8 binding to the amino-terminal portion of each protein; thus, the first 58 amino acids of the high affinity receptor conferred high affinity binding properties to the low affinity receptor, and the first 62 amino acids of the low affinity receptor conferred low affinity binding properties to the high affinity receptor. These results suggest that the high affinity IL-8 binding domain is contained in the amino terminus of the F3R protein and the low affinity IL-8 binding domain is contained in the amino terminus of the 4AB protein. Interestingly, the F3R/4AB chimera bound IL-8 more strongly than either the F3R or the 4AB receptor, indicating that interaction(s) between the amino-terminal binding domain and other portions of the molecule may occur.

Binding of ligand by the amino terminus of the IL-8 receptor was also suggested by the experiment depicted in Fig. 8. COS cells were transiently transfected with 4AB-pSVL or F3R/4AB-pSVL and harvested and washed as described above. 1.2μM ^{125}I-labelled IL-8 was added to a mixture of 2 x 10^5 whole cells (in 50μl PBS/0.1% BSA) and increasing concentrations f competing ligand (i.e., between 0 and 1000nM unlabelled IL-8 or b tween 10 and 500nM MGSA/GROα). C lls were incubated with ligand for

one hour at 4°C, filt red thr ugh GF/C filters which had be n soaked in 0.3% PEI, rinsed with cold PBS/0.1% BSA, and the cell-bound radioactivity measured in a gamma counter.

As shown in Fig. 8, the 4AB receptor bound IL-8 and MGSA/GROα with similar affinities. In contrast, with the F3R/4AB receptor (i.e., the receptor including the putative F3R IL-8 binding domain), the binding of IL-8 could not be competed with MGSA/GROα. This is characteristic of F3R-mediated IL-8 binding. Thus, the extracellular N-terminal domain of the high affinity IL-8 receptor confers both high affinity and specificity.

Screening For IL-8 Receptor Antagonists

As discussed above, one aspect of the invention features screening for compounds that antagonize the interaction between IL-8 and the IL-8 receptor, thereby preventing or reducing the cascade of events that are mediated by that interaction. The elements of the screen are IL-8 and recombinant IL-8 receptor (or a suitable receptor fragment or analog, as outlined above) configured to permit detection of binding. As described above, IL-8 may be purchased from Genzyme and a full-length rabbit or human IL-8 receptor (or an IL-8-binding fragment or analog) may be produced as described herein.

Binding of IL-8 to its receptor may be assayed by any of the methods described above. Preferably, cells expressing recombinant IL-8 receptor (or a suitable IL-8 receptor fragment or analog) are immobilized on a solid substrate (e.g., the well of a microtiter plate or a column) and reacted with detectably-labelled IL-8 (as described above). Binding is assayed by the detection label in association with the receptor component (and, therefore, in association with the solid substrate). Binding of labelled IL-8 to receptor-bearing c lls is used as a "control" against which antagonist assays are

measured. The antagonist assays involve incubati n of the IL-8 receptor-bearing cells with an appropriate amount of candidate antagonist. To this mix, an equivalent amount of labelled IL-8 is added. An antagonist useful in the invention specifically interferes with labelled IL-8 binding to the immobilized receptor-expressing cells.

An antagonist is then tested for its ability to interfere with IL-8 function, i.e., to specifically interfere with labelled IL-8 binding without resulting in signal transduction normally mediated by the receptor. To test this using a functional assay, stably transfected cell lines containing the IL-8 receptor can be produced as described herein and reporter compounds such as the calcium binding agent, FURA-2, loaded into the cytoplasm by standard techniques. Stimulation of the heterologous IL-8 receptor with IL-8 or another agonist leads to intracellular calcium release and the concomitant fluorescence of the calcium-FURA-2 complex. This provides a convenient means for measuring agonist activity. Inclusion of potential antagonists along with IL-8 allows for the screening and identification of authentic receptor antagonists as those which effectively block IL-8 binding without producing fluorescence (i.e., without causing the mobilization of intracellular Ca^{++}). Such an antagonist may be expected to be a useful therapeutic agent for inflammatory disorders.

Appropriate candidate antagonists include IL-8 receptor fragments, particularly fragments containing an IL-8-binding portion adjacent to or including one or more transmembrane segments 2-7 or an extracellular domain of the receptor (described above); such fragments would preferably including five or more amin acids. Other candidate antagonists include analogs of IL-8 and other peptides as well as n n-peptid compounds and anti-IL-8

r ceptor antibodies d signed or derived from analysis of the r cept r.

Anti IL-8 Receptor Antibodies

High affinity or low affinity IL-8 receptors (or immunogenic receptor fragments or analogs) may be used to raise antibodies useful in the invention. As described above, receptor fragments preferred for the production of antibodies are those fragments deduced or shown experimentally to be extracellular; such fragments include the extracellular N-terminal domain.

Antibodies directed to IL-8 receptor peptides are produced as follows. Peptides corresponding to all or part of the putative extracellular loops (approximately amino acids 94-113, 186-202, and 268-285 of the high affinity IL-8 receptor or approximately amino acids 107-120, 184-213, and 274-300 of the low affinity IL-8 receptor) or to all or a portion of the extracellular N-terminal domain (approximately amino acids 1-37 of the high affinity IL-8 receptor or approximately amino acids 1-50 of the low affinity IL-8 receptor) are produced using a peptide synthesizer, by standard techniques. The peptides are coupled to KLH with m-maleimide benzoic acid N-hydroxysuccinimide ester. The KLH-peptide is mixed with Freund's adjuvant and injected into animals, e.g. guinea pigs or goats. Antibodies are purified by peptide antigen affinity chromatography. Using such a method, polyclonal antisera were raised to peptides which included the N-terminal extracellular domain and also to loops 2 and 3.

Additional peptides used for immunizations were the following:

1. Amino acids 16-39 of human IL-8 receptor: NFTGMPPADEDYSPCMLETE-TLNK(c) (Cys added for conjugation). (See Holmes et al. 1991, Sci nce, Vol. 253:1278 for s qu nce.)

2. Three peptid s from rabbit "high affinity" IL-8 receptor; human "low affinity" receptor, and rabbit "low affinity" receptor: amino acids 21-44, 21-49, and 21-46 respectively. The internal cysteine at positions 35, 39 and 37 (respectively) have been replaced with alanines, and a cysteine was added to COOH-terminus for conjugation.

Alternatively, antibodies to the IL-8 receptor are produced using whole cells expressing the IL-8 receptor, or membrance fractions of these cells (both described above). For example, approximately 10^7 transiently transfected COS7 cells, stably transfected CHO cells, or membrane fragments corresponding to 50 μg total membrane protein are injected into mice. After 2 weeks and 4 weeks the animals are boosted with approximately 10^7 cells or membrane fragments corresponding to 10-25 μg protein. Approximately 3 weeks following the second boost, the animals are boosted once again, and spleen cells are removed for the making of hybridomas using standard techniques. Hybridomas producing antibodies that bind to the IL-8 receptor are screened by FACS (flourescence activated cell sorter), by cell-based ELISA using untransfected versus transfected cells (preferably of a cell type different from the cells used in the immunization), or using membranes. Hybridomas producing antibodies that bind to tranfected cells are subcloned and tested for ability to block IL-8 binding to the receptor, or to block IL-8 dependent signal transduction.

Once produced, antibodies are tested for specific IL-8 receptor recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize the IL-8 receptor are considered to be likely candidates for useful antagonists; such candidates are further tested for th ir ability to specifically

interf re with the interaction betwe n IL-8 and its receptor (as described above). Antibodies which antagonize IL-8/IL-8 receptor binding or IL-8 receptor function are considered to be useful as antagonists in the invention.

Therapy

Particularly suitable therapeutics for the treatment of inflammatory diseases are the soluble antagonistic receptor fragments described above formulated in an appropriate buffer such as physiological saline. Where it is particularly desirable to mimic the receptor conformation at the membrane interface, the fragment may include a sufficient number of adjacent transmembrane residues. In this case, the fragment may be associated with an appropriate lipid fraction (e.g., in lipid vesicles or attached to fragments obtained by disrupting a cell membrane). Alternatively, anti-IL-8 receptor antibodies produced as described above may be used as a therapeutic. Again, the antibodies would be administered in a pharmaceutically-acceptable buffer (e.g., physiological saline). If appropriate, the antibody preparation may be combined with a suitable adjuvant.

The therapeutic preparation is administered in accordance with the condition to be treated. Ordinarily, it will be administered intravenously, at a dosage that provides suitable competition for IL-8 binding. Alternatively, it may be convenient to administer the therapeutic orally, nasally, or topically, e.g., as a liquid or a spray. Again, the dosages are as described above. Treatment may be repeated as necessary for alleviation of disease symptoms. Antagonists may also be administered to prevent (as well as treat) inflammation; the antagonist is administ r d as d scribed above.

Because the IL-8 receptor is involved in neutrophil activation associated with inflammation, IL-8 receptor antagonists can be used to treat or prevent any inflammatory disease in which neutrophils play a principal role, such as psoriasis, rheumatoid arthritis, and other chronic disorders as well as acute inflammatory disorders such as reperfusion injury, septic shock, trauma shock, and pulmonary disorders such as adult respiratory distress syndrome (ARDS) and inflammatory airway disorders caused by bacterial infections in cystic fibrosis patients.

The methods of the invention may be used to reduce inflammatory responses in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated, the IL-8 receptor or receptor fragment or analog or the antibody employed is preferably specific for that species.

Other embodiments are within the claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Navarro, Javier et al.

(ii) TITLE OF INVENTION: INTERLEUKIN-8 RECEPTORS AND RELATED MOLECULES AND METHODS

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: July 9, 1991
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/685,101
(B) FILING DATE: April 10, 1991

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00231/051002

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1200
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 1

```
CCGGCNTCNG AGCAGCTGAA GCTTGCATGC CTGCAGGTCG ACTCTAGAGG ACCCCCGGGT      60

ACCGAGCTCG AATTCAGCTC CGATCTTAAG GTGAAACTGT GGCCGTA                    107

ATG GAA GTA AAC GTA TGG AAT ATG ACT GAT CTG TGG ACG TGG TTT GAG        155
Met Glu Val Asn Val Trp Asn Met Thr Asp Leu Trp Thr Trp Phe Glu
                5                  10                  15

GAT GAG TTT GCA AAT GCT ACT GGT ATG CCT CCT GTA GAA AAA GAT TAT        203
Asp Glu Phe Ala Asn Ala Thr Gly Met Pro Pro Val Glu Lys Asp Tyr
            20                  25                  30

AGC CCC TGT CTG GTA GTC ACC CAG ACA CTT AAC AAA TAT GTT GTG GTC        251
Ser Pro Cys Leu Val Val Thr Gln Thr Leu Asn Lys Tyr Val Val Val
        35                  40                  45

GTC ATC TAT GCC CTG GTC TTC CTG CTG AGC CTG CTG GGC AAC TCC CTG        299
Val Ile Tyr Ala Leu Val Phe Leu Leu Ser Leu Leu Gly Asn Ser Leu
    50                  55                  60

GTG ATG CTG GTC ATA CTG TAC AGC CGG AGC AAC CGT TCG GTC ACC GAC        247
Val Met Leu Val Ile Leu Tyr Ser Arg Ser Asn Arg Ser Val Thr Asp
65                  70                  75                  80

GTC TAC CTG CTG AAC CTG GCC ATG GCC GAC CTG CTT TTT GCC CTG ACC        395
Val Tyr Leu Leu Asn Leu Ala Met Ala Asp Leu Leu Phe Ala Leu Thr
                85                  90                  95

ATG CCT ATC TGG GCC  TC TCC AAG GAA AAA GGC TGG ATT TTC GGC ACG        443
Met Pro Ile Trp Ala Val Ser Lys Glu Lys Glu Trp Ile Phe Gly Thr
            100                 105                 110
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CCC CTG TGC AAG GTG GGG TCG CTT GTG AAG GAA GTC AAC TTC TAC AGT                491
Pro Leu Cys Lys Val Val Ser Leu Val Lys Glu Val Asn Phe Tyr Ser
        115                 120                 125

GAA ATC CTG CTC CTG GCC TGC ATC AGT GTG GAC CGC TAC CTG GCC ATT                539
Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp Arg Tyr Leu Ala Ile
    130                 135                 140

GTC CAT GCT ACT CGC ACA CTG ACC CAG AAG CGC CAC TTG GTC AAG TTC                587
Val His Ala Thr Arg Thr Leu Thr Gln Lys Arg His Leu Val Lys Phe
145                 150                 155                 160

ATA TGT CTG GGC ATC TGG GCG CTG TCT CTG ATT TTG TCC CTG CCC TTC                635
Ile Cys Leu Gly Ile Trp Ala Leu Ser Leu Ile Leu Ser Leu Pro Phe
                165                 170                 175

TTC CTC TTC CGC CAA GTC TTT TCT CCA AAC AAT TCC AGC CCG GTC TGC                683
Phe Leu Phe Arg Gln Val Phe Ser Pro Asn Asn Ser Ser Pro Val Cys
            180                 185                 190

TAT GAG GAC CTG GGT CAC AAC ACA GCG AAA TGG CGC ATG GTG CTG CGG                731
Tyr Glu Asp Leu Gly His Asn Thr Ala Lys Trp Arg Met Val Leu Arg
        195                 200                 205

ATC CTG CCA CAC ACT TTC GGC TTC ATC CTG CCG CTG CTG GTC ATG CTG                779
Ile Leu Pro His Thr Phe Gly Phe Ile Leu Pro Leu Leu Val Met Leu
    210                 215                 220

TTT TGC TAT GGG TTC ACC CGT CGC ACG CTG TTC CAG GCC CAC ATG GGG                827
Phe Cys Tyr Gly Phe Tyr Leu Arg Thr Leu Phe Gln Ala His Met Gly
225                 230                 235                 240

CAG AAG CAC CGG GCC ATG CGG GTC ATC TTC GCC GTC GTG CTC ATC TTC                875
Gln Lys His Arg Ala Met Arg Val Ile Phe Ala Val Val Leu Ile Phe
                245                 250                 255

CTT CTC TGC TGG CTG CCC TAC AAC CTG GTC CTG CTC GCA GAC ACC CTC                923
Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu Leu Ala Asp Thr Leu
            260                 265                 270

ATG AGG ACC CAC GTG ATC CAG GAG ACG TGT CAG CGT CGC AAT GAC ATT                971
Met Arg Thr His Val Ile Gln Glu Thr Cys Gln Arg Arg Asn Asp Ile
        275                 280                 285

GAC CGG GCC CTG GAC GCC ACC GAG ATT CTG GGC TTC CTG CAC AGC TGC               1019
Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly Phe Leu His Ser Cys
    290                 295                 300

CTC AAC CCC ATC ATC TAC GCC TTC ATT GGC CAA AAC TTT CGC AAT GGA               1067
Leu Asn Pr  Ile Ile Tyr Ala Phe Ile Gly Gln Asn Phe Arg Asn Gly
305                 310                 315                 320
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```
TTC CTC AAG ATG CTT GCG GCC CGC GGC CTT ATT AGC AAG GAG TTC CTG          1115
Phe Leu Lys Met Leu Ala Ala Arg  ly Leu Ile Ser Lys Glu Phe Leu
                325                 330                 335

ACA CGA CAT CGG GTC ACC TCT TAT ACT TCT TCC TCT ACC AAC GTG CCT          1163
Thr Arg His Arg Val Thr Ser Tyr Thr Ser Ser Ser Thr Asn Val Pro
            340                 345                 350

TCA AAT CTC                                                              1172
Ser Asn Leu
        355

TAAAGCCATC TGTGAAAGAC TGCCTCCC                                           1200
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	42
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 2

CATGATNAGG TCNGCNCAGG CCAGGCTCAG CAGGAAGTAG TT 42

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	24
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 3

GAATATGGGG AATTTATTAT GCAG 24

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	25
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 4

AATGTGACTG TGAAGAGAAG GGAGG 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1106
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 5

```
TTTACCTCAA AA                                                          12

ATG GAA GAT TTT AAC ATG GAG AGT GAC AGC TTT GAA GAT TTC TGG AAA        60
Met Glu Asp Phe Asn Met Glu Ser Asp Ser Phe Glu Asp Phe Trp Lys
                  5                  10                  15

GGT GAA GAT CTT AGT AAT TAC AGT TAC AGC TCT ACC CTG CCC CCT TTT       108
Gly Glu Asp Leu Ser Asn Tyr Ser Tyr Ser Ser Thr Leu Pro Pro Phe
            20                  25                  30

CTA CTA GAT GCC GCC CCA TGT GAA CCA GAA TCC CTG GAA ATC AAC AAG       156
Leu Leu Asp Ala Ala Pro Cys Glu Pro Glu Ser Leu Glu Ile Asn Lys
        35                  40                  45

TAT TTT GTG GTC ATT ATC TAT GCC CTG GTA TTC CTG CTG AGC CTG CTG       204
Tyr Phe Val Val Ile Ile Tyr Ala Leu Val Phe Leu Leu Ser Leu Leu
    50                  55                  60

GGA AAC TCC CTC GTG ATG CTG GTC ATC TTA TAC AGC AGG GTC GGC CGC       252
Gly Asn Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg Val Gly Arg
65                  70                  75                  80

TCC GTC ACT GAT GTC TAC CTG CTG AAC CTA GCC TTG GCC GAC CTA CTC       300
Ser Val Thr Asp Val Tyr Leu Leu Asn Leu Ala Leu Ala Asp Leu Leu
                85                  90                  95

TTT GCC CTG ACC TTG CCC ATC TGG GCC GCC TCC AAG GTG AAT GCC TGG       348
Phe Ala Leu Thr Leu Pro Ile Trp Ala Ala Ser Lys Val Asn Gly Trp
            100                 105                 110

ATT TTT GGC ACA TTC CTG TGC AAG GTG GTC TCA CTC CTG AAG GAA GTC       396
Ile Phe Gly Thr Phe Leu Cys Lys Val Val Ser Leu Leu Lys Glu Val
        115                 120                 125
```

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AAC TTC TAT AGT GGC ATC CTG CTA CTG GCC TGC ATC AGT GTG GAC CGT          444
Asn Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp Arg
    130                 135                 140

TAC CTG GCC ATT GTC CAT GCC ACA CGC ACA CTG ACC CAG AAG GCG TAC          492
Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu Thr Gln Lys Arg Tyr
145                 150                 155                 160

TTG GTC AAA TTC ATA TGT CTC AGC ATC TGG GTT CTG TCC TTG CTC CTG          540
Leu Val Lys Phe Ile Cys Leu Ser Ile Trp Gly Leu Ser Leu Leu Leu
                165                 170                 175

GCC CTG CCT GTC TTA CTT TTC GCA AGG ACC GTC TAC TCA TCC AAT GTT          588
Ala Leu Pro Val Leu Leu Phe Arg Arg Thr Val Tyr Ser Ser Asn Val
            180                 185                 190

AGC CCA GCC TGC TAT GAG GAC ATG GGC AAC AAT ACA GCA AAC TGG GCC          636
Ser Pro Ala Cys Tyr Glu Asp Met Gly Asn Asn Thr Ala Asn Trp Arg
        195                 200                 205

ATG CTG TTA GCC ATC CTG CCC CAG TCC TTT GGC TTC ATC GTG CCA CTG          684
Met Leu Leu Arg Ile Leu Pro Gln Ser Phe Gly Phe Ile Val Pro Leu
    210                 215                 220

CTG ATC ATG CTG TTC TGC TAC GGA TTC ACC CTG CGT ACG CTG TTT AAG          732
Leu Ile Met Leu Phe Cys Tyr Gly Phe Thr Leu Arg Thr Leu Phe Lys
225                 230                 235                 240

GCC CAC ATG GGG CAG AAG CAC CGG GCC ATG CGG GTC ATC TTT GCT GTC          780
Ala His Met Gly Gln Lys His Arg Ala Met Arg Val Ile Phe Ala Val
                245                 250                 255

GTC CTC ATC TTC CTG CTT TGC TGG CTG CCC TAC AAC CTG GTC CTG CTG          828
Val Leu Ile Phe Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu Leu
            260                 265                 270

GCA GAC ACC CTC ATG AGG ACC CAG GTG ATC CAG GAG ACC TGT GAG CGC          876
Ala Asp Thr Leu Met Arg Thr Gln Val Ile Gln Glu Thr Cys Glu Arg
        275                 280                 285

CGC AAT CAC ATC GAC CGG GCT CTG GAT GCC ACC GAG ATT CTG GGC ATC          924
Arg Asn His Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly Ile
    290                 295                 300

CTT CAC AGC TGC CTC AAC CCC CTC ATC TAC GCC TTC ATT GGC CAG AAG          972
Leu His Ser Cys Leu Asn Pro Leu Ile Tyr Ala Phe Ile Gly Gln Lys
305                 310                 315                 320

TTT CGC CAT GGA CTC CTC AAG ATT CTA GCT ATA CAT GGC TTG ATC AGC         1020
Phe Arg His  ly Leu Leu Lys Ile Leu Ala Ile His Gly Leu Ile Ser
                325                 330                 335
```

```
AA  GAC TCC CTG CCC AAA GAC AGC AGG CCT TCC TTT GTT GGC TCT TCT      1068
Lys Asp Ser Leu Pro Lys Asp Ser Arg Pro Ser Phe Val Gly Ser Ser
            340                 345                 350

TCA GGG CAC ACT TCC ACT ACT CTC                                    1092
Ser Gly His Thr Ser Thr Thr Leu
        355                 360

TAAGACCTCC TGCC                                                    1106
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1373
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 6

```
GGGAATTCCG CCAGCCCGCT CACAGGCAGT GGCTGTCGCA GCAACAGCAG GATTTAAGAC    60

TATCTCAGAA                                                           70

ATG CAA GAG TTT ACC TGG GAG AAT TAC AGC TAT GAA GAT TTT TTC GGC      118
Met Gln Glu Phe Thr Trp Glu Asn Tyr Ser Tyr Glu Asp Phe Phe Gly
                5                   10                  15

GAT TTC AGC AAT TAC AGT TAC AGC ACT GAC CTA CCC CCT ACC CTG CTA      166
Asp Phe Ser Asn Tyr Ser Tyr Ser Thr Asp Leu Pro Pro Thr Leu Leu
            20                  25                  30

GAC TCT GCT CCG TGC CGG TCA GAA TCT CTG GAA ACC AAC AGC TAT GTT      214
Asp Ser Ala Pro Cys Arg Ser Gly Ser Leu Glu Thr Asn Ser Tyr Val
        35                  40                  45

GTG CTC ATC ACC TAT ATC CTG GTC TTC CTG CTG AGC CTG CTG GGC AAC      262
Val Leu Ile Thr Tyr Ile Leu Val Phe Leu Leu Ser Leu Leu Gly Asn
    50                  55                  60

TCC CTG GTG ATG CTG GTC ATC CTG TAC AGC CGG AGC ACC TGC TCG GTC      310
Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg Ser Thr Cys Ser Val
65                  70                  75                  80

ACC GAC GTC TAC CTG CTG AAC CTG GCC ATC GCC GAC CTG CTC TTT GCC      358
Thr Thr Leu Pro Ile Trp Ala Ala Ser Lys Val His Gly Trp Thr Phe
                85                  90                  95
```

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ACC ACC TTG CCC ATC TGG GCC GCC TCC AAG GTG CAC GGC TGG ACT TTC          406
Thr Thr Leu Pro Il  Trp Ala Ala Ser Lys Val His Gly Trp Thr Ph
            100                 105                 110

GGC ACG CCC CTG TGT AAG GTG GTC TCG CTT GTG AAG GAA GTC AAC TTC          454
Gly Thr Pro Leu Cys Lys Val Val Ser Leu Val Lys Glu Val Asn Phe
        115                 120                 125

TAC AGC GGA ATC CTG CTC CTG GCC TGC ATC AGT GTG GAC CGC TAC CTG          502
Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp Arg Tyr Leu
    130                 135                 140

GCC ATC GTC CAT GCC ACA CGC ACG ATG ATC CAG AAG CGC CAC TTG GTC          550
Ala Ile Val His Ala Thr Arg Thr Mat Ile Gln Lys Arg His Leu Val
145                 150                 155                 160

AAG TTC ATA TGC TTA AGC ATG TGG GGA GTG TCT TTG ATC CTG TCT CTG          598
Lys Phe Ile Cys Leu Ser Met Trp Gly Val Ser Leu Ile Leu Ser Leu
                165                 170                 175

CCC ATC TTA CTG TTC CGT AAT GCC ATC TTC CCA CCC AAT TCC AGC CCG          646
Pro Ile Leu Leu Phe Arg Asn Ala Ile Phe Pro Pro Asn Ser Ser Pro
            180                 185                 190

GTC TGC TAT GAG GAC ATG GGG AAC AGC ACT GCG AAA TGG CGC ATG GTG          694
Val Cys Tyr Glu Asp Met Gly Asn Ser Thr Ala Lys Trp Arg Met Val
        195                 200                 205

CTG CGG ATC CTG CCT CAG ACT TTC GGC TTC ATC CTG CCG CTG CTG GTC          742
Leu Arg Ile Leu Pro Gln Thr Phe Gly Phe Ile Leu Pro Leu Leu Val
    210                 215                 220

ATG CTG TTT TGC TAT GTG TTC ACC CTG CGC ACG CTG TTC CAG GCC CAC          790
Mat Leu Phe Cys Tyr Val Phe Thr Leu Arg Thr Leu Phe Gln Ala His
225                 230                 235                 240

ATG GGG CAG AAG CAC CGG GCC ATG CGG GTC ATC TTC GCC GTC GTG CTC          838
Met Gly Gln Lys His Arg Ala Met Arg Val Ile Phe Ala Val Val Leu
                245                 250                 255

ATC TTC CTT CTC TGT TGG CTG CCC TAC AAC CTG GTT CTG CTC ACA GAC          886
Ile Phe Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu Leu Thr Asp
            260                 265                 270
```

```
ACC CTC ATG AGG ACC CAC GTG ATC CAG GAG ACG TGT GAG CGC CGC AAT          934
Thr Leu Met Arg Thr His Val Ile  ln Glu Thr Cys Glu Arg Arg Asn
        275                 280                 285

GAC ATT GAC CGG GCC CTG GAC GCC ACC GAG ATT CTG GGC TTC CTG CAC          982
Asp Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly Phe Leu His
    290                 295                 300

AGC TGC CTC AAC CCC ATC ATC TAC GCC TTC ATT GGG CAA AAG TTT CGC         1030
Ser Cys Leu Asn Pro Ile Ile Tyr Ala Phe Ile Gly Gln Lys Phe Arg
305                 310                 315                 320

TAT GGC CTG CTC AAG ATC CTG GCG GCC CAC GGC CTG ATC AGC AAG GAG         1078
Tyr Gly Leu Leu Lys Ile Leu Ala Ala His Gly Leu Ila Ser Lys Glu
                325                 330                 335

TTC CTG GCC AAG GAG AGC AGG CCT TCC TTT GTC GCC TCG TCT TCA GGG         1126
Phe Leu Ala Lys Glu Ser Arg Pro Ser Phe Val Ala Ser Ser Ser Gly
            340                 345                 350

AAC ACC TCT ACC ACC CTC                                                 1144
Asn Thr Ser Thr Thr Leu
        355

TAAGACGCCT ATGTGGGCTG CAGTCTCTCG GGCTTCCTCC CTCCCTTGGA CATCTCATCC       1204

CAAGNCTCAT ATCCTGGTCC CGGAGTCAAC ACAGTCCTCA CTGTGGTTAT AGAAAAGAGC       1264

GGNGGGCACT TCCTCAGTAG GTCCCCAGTG TACAGNTTAG AAAGNCTGAT CCGGNCCCTG       1324

TCACTTCCCA TAATTACTCT NTCAACTACG GGAATCTTCT CATTTCTAC                    1373
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	23
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 7

```
GGGAAACTCC CTCGTGATGC TGG                                               23
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	26
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO. 8

GTCTGCCAGC AGGACCAGGT TGTAGG 26

Claims

1. Recombinant mammalian IL-8 receptor polypeptide.

2. The polypeptide of claim 1, comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 1 (SEQ ID NO:1).

3. The polypeptide of claim 1, comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 2 (SEQ ID NO: 5)

4. The polypeptide of claim 1, comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 9 (SEQ ID NO:6).

5. A substantially isolated polypeptide which is a fragment or analog of an IL-8 receptor comprising a domain capable of binding IL-8.

6. The polypeptide of claim 5, said polypeptide comprising amino acids 1-37 of the amino acid sequence shown in Fig. 1 (SEQ ID NO.:1), or an IL-8 binding fragment thereof.

7. The polypeptide of claim 5, said polypeptide comprising amino acids 1-50 of the amino acid sequence shown in Fig. 2 (SEQ ID NO.:5), or an IL-8 binding fragment thereof.

8. Purified DNA which encodes a polypeptide of claim 1 or claim 5.

9. The purified DNA of claim 8, wherein said DNA is cDNA.

10. A cell which contains the purified DNA of claim 8.

11. A method of producing a recombinant IL-8 receptor polypeptide or a fragment or analog thereof, comprising

providing a cell transformed with DNA encoding the IL-8 receptor or a fragment or analog thereof positioned for expression is said cell;

culturing said transformed cell under conditions for expressing said DNA; and

isolating said recombinant IL-8 receptor polypeptide.

12. A purified antibody which binds preferentially to a polypeptide of claims 1 or 5.

13. The antibody of claim 12, wherein said antibody neutralizes the biological activity in vivo of said polypeptide.

14. A therapeutic composition comprising as an active ingredient a polypeptide according to claims 1 or 5, said active ingredient being formulated in a physiologically-acceptable carrier.

15. A therapeutic composition comprising as an active ingredient an antibody according to claim 12, said active ingredient being formulated in a physiologically-acceptable carrier.

16. A method of screening candidate compounds for the ability to antagonize interaction between IL-8 and an IL-8 rec ptor, said method comprising:

a) mixing a candidate antagonist compound with a first compound comprising a recombinant IL-8 receptor polypeptide of claim 1 or a receptor fragment or analog of claim 5 on the one hand and with a second compound comprising IL-8;

b) determining whether said first and second compounds bind; and

c) identifying antagonistic compounds as those which interfere with the binding of the first compound to the second compound and which reduce the IL-8-mediated release of intracellular Ca^{++}.

17. A polypeptide comprising an amino-terminal portion of the sequence shown in Fig. 1 (SEQ ID NO.:1) fused to a carboxy-terminal portion of the sequence shown in Fig. 2 (SEQ ID NO.:5).

```
CCGGCNTCNG AGCAGCTGAA GCTTGCATGC CTGCAGGTCG ACTCTAGAGG ATCCCCGGGT

ACCGAGCTCG AATTCAGCTC CGATCTTAAG GTGAAACTGT GGCCGTA ATG GAA GTA
                                                    Met Glu Val

AAC GTA TGG AAT ATG ACT GAT CTG TGG ACG TGG TTT GAG GAT GAG TTT
Asn Val Trp Asn Met Thr Asp Leu Trp Thr Trp Phe Glu Asp Glu Phe

GCA AAT GCT ACT GGT ATG CCT CCT GTA GAA AAA GAT TAT AGC CCC TGT
Ala Asn Ala Thr Gly Met Pro Pro Val Glu Lys Asp Tyr Ser Pro Cys

CTG GTA GTC ACC CAG ACA CTT AAC AAA TAT GTT GTG GTC GTC ATC TAT
Leu Val Val Thr Gln Thr Leu Asn Lys Tyr Val Val Val Val Ile Tyr

GCC CTG GTC TTC CTG CTG AGC CTG CTG GGC AAC TCC CTG GTG ATG CTG
Ala Leu Val Phe Leu Leu Ser Leu Leu Gly Asn Ser Leu Val Met Leu

GTC ATA CTG TAC AGC CGG AGC AAC CGT TCG GTC ACC GAC GTC TAC CTG
Val Ile Leu Tyr Ser Arg Ser Asn Arg Ser Val Thr Asp Val Tyr Leu

CTG AAC CTG GCC ATG GCC GAC CTG CTT TTT GCC CTG ACC ATG CCT ATC
Leu Asn Leu Ala Met Ala Asp Leu Leu Phe Ala Leu Thr Met Pro Ile

TGG GCC GTC TCC AAG GAA AAA GGC TGG ATT TTC GGC ACG CCC CTG TGC
Trp Ala Val Ser Lys Glu Lys Gly Trp Ile Phe Gly Thr Pro Leu Cys

AAG GTG GTC TCG CTT GTG AAG GAA GTC AAC TTC TAC AGT GGA ATC CTG
Lys Val Val Ser Leu Val Lys Glu Val Asn Phe Tyr Ser Gly Ile Leu

CTC CTG GCC TGC ATC AGT GTG GAC CGC TAC CTG GCC ATT GTC CAT GCT
Leu Leu Ala Cys Ile Ser Val Asp Arg Tyr Leu Ala Ile Val His Ala

ACT CGC ACA CTG ACC CAG AAG CGC CAC TTG GTC AAG TTC ATA TGT CTG
Thr Arg Thr Leu Thr Gln Lys Arg His Leu Val Lys Phe Ile Cys Leu

GGC ATC TGG GCG CTG TCT CTG ATT TTG TCC CTG CCC TTC TTC CTC TTC
Gly Ile Trp Ala Leu Ser Leu Ile Leu Ser Leu Pro Phe Phe Leu Phe

CGC CAA GTC TTT TCT CCA AAC AAT TCC AGC CCG GTC TGC TAT GAG GAC
Arg Gln Val Ph  Ser Pr  Asn Asn Ser S r Pro Val Cys Tyr Glu Asp
```

FIG. 1

(PAGE 1 OF 2)

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CTG GGT CAC AAC ACA GCG AAA TGG CGC ATG GTG CTG CGG ATC CTG CCA
Leu Gly His Asn Thr Ala Lys Trp Arg Met Val Leu Arg Ile Leu Pro

CAC ACT TTC GGC TTC ATC CTG CCG CTG CTG GTC ATG CTG TTT TGC TAT
His Thr Phe Gly Phe Ile Leu Pro Leu Leu Val Met Leu Phe Cys Tyr

GGG TTC ACC CTG CGC ACG CTG TTC CAG GCC CAC ATG GGG CAG AAG CAC
Gly Phe Thr Leu Arg Thr Leu Phe Gln Ala His Met Gly Gln Lys His

CGG GCC ATG CGG GTC ATC TTC GCC GTC GTG CTC ATC TTC CTT CTC TGC
Arg Ala Met Arg Val Ile Phe Ala Val Val Leu Ile Phe Leu Leu Cys

TGG CTG CCC TAC AAC CTG GTC CTG CTC GCA GAC ACC CTC ATG AGG ACC
Trp Leu Pro Tyr Asn Leu Val Leu Leu Ala Asp Thr Leu Met Arg Thr

CAC GTG ATC CAG GAG ACG TGT CAG CGT CGC AAT GAC ATT GAC CGG GCC
His Val Ile Gln Glu Thr Cys Gln Arg Arg Asn Asp Ile Asp Arg Ala

CTG GAC GCC ACC GAG ATT CTG GGC TTC CTG CAC AGC TGC CTC AAC CCC
Leu Asp Ala Thr Glu Ile Leu Gly Phe Leu His Ser Cys Leu Asn Pro

ATC ATC TAC GCC TTC ATT GGC CAA AAC TTT CGC AAT GGA TTC CTC AAG
Ile Ile Tyr Ala Phe Ile Gly Gln Asn Phe Arg Asn Gly Phe Leu Lys

ATG CTT GCG GCC CGC GGC CTT ATT AGC AAG GAG TTC CTG ACA CGA CAT
Met Leu Ala Ala Arg Gly Leu Ile Ser Lys Glu Phe Leu Thr Arg His

CGG GTC ACC TCT TAT ACT TCT TCC TCT ACC AAC GTG CCT TCA AAT CTC
Arg Val Thr Ser Tyr Thr Ser Ser Ser Thr Asn Val Pro Ser Asn Leu

TAAAGCCATC TGTGAAAGAC TGCCTCCC
```

FIG. 1

(PAGE 2 OF 2)

```
TTTACCTCAA AA ATG GAA GAT TTT AAC ATG GAG AGT GAC AGC TTT GAA GAT
              Met Glu Asp Phe Asn Met Glu Ser Asp Ser Phe Glu Asp

TTC TGG AAA GGT GAA GAT CTT AGT AAT TAC AGT TAC AGC TCT ACC CTG
Phe Trp Lys Gly Glu Asp Leu Ser Asn Tyr Ser Tyr Ser Ser Thr Leu

CCC CCT TTT CTA CTA GAT GCC GCC CCA TGT GAA CCA GAA TCC CTG GAA
Pro Pro Phe Leu Leu Asp Ala Ala Pro Cys Glu Pro Glu Ser Leu Glu

ATC AAC AAG TAT TTT GTG GTC ATT ATC TAT GCC CTG GTA TTC CTG CTG
Ile Asn Lys Tyr Phe Val Val Ile Ile Tyr Ala Leu Val Phe Leu Leu

AGC CTG CTG GGA AAC TCC CTC GTG ATG CTG GTC ATC TTA TAC AGC AGG
Ser Leu Leu Gly Asn Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg

GTC GGC CGC TCC GTC ACT GAT GTC TAC CTG CTG AAC CTA GCC TTG GCC
Val Gly Arg Ser Val Thr Asp Val Tyr Leu Leu Asn Leu Ala Leu Ala

GAC CTA CTC TTT GCC CTG ACC TTG CCC ATC TGG GCC GCC TCC AAG GTG
Asp Leu Leu Phe Ala Leu Thr Leu Pro Ile Trp Ala Ala Ser Lys Val

AAT GGC TGG ATT TTT GGC ACA TTC CTG TGC AAG GTG GTC TCA CTC CTG
Asn Gly Trp Ile Phe Gly Thr Phe Leu Cys Lys Val Val Ser Leu Leu

AAG GAA GTC AAC TTC TAT AGT GGC ATC CTG CTA CTG GCC TGC ATC AGT
Lys Glu Val Asn Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser

GTG GAC CGT TAC CTG GCC ATT GTC CAT GCC ACA CGC ACA CTG ACC CAG
Val Asp Arg Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu Thr Gln

AAG CGC TAC TTG GTC AAA TTC ATA TGT CTC AGC ATC TGG GGT CTG TCC
Lys Arg Tyr Leu Val Lys Phe Ile Cys Leu Ser Ile Trp Gly Leu Ser

TTG CTC CTG GCC CTG CCT GTC TTA CTT TTC CGA AGG ACC GTC TAC TCA
Leu Leu Leu Ala Leu Pro Val Leu Leu Phe Arg Arg Thr Val Tyr Ser

TCC AAT GTT AGC CCA GCC TGC TAT GAG GAC ATG GGC AAC AAT ACA GCA
S r Asn Val Ser Pro Ala Cys Tyr Glu Asp M t Gly Asn Asn Thr Ala
```

FIG. 2
(PAGE 1 OF 2)

```
AAC TGG CGG ATG CTG TTA CGG ATC CTG CCC CAG TCC TTT GGC TTC ATC
Asn Trp Arg Met Leu Leu Arg Ile Leu Pro Gln Ser Phe Gly Phe Ile

GTG CCA CTG CTG ATC ATG CTG TTC TGC TAC GGA TTC ACC CTG CGT ACG
Val Pro Leu Leu Ile Met Leu Phe Cys Tyr Gly Phe Thr Leu Arg Thr

CTG TTT AAG GCC CAC ATG GGG CAG AAG CAC CGG GCC ATG CGG GTC ATC
Leu Phe Lys Ala His Met Gly Gln Lys His Arg Ala Met Arg Val Ile

TTT GCT GTC GTC CTC ATC TTC CTG CTT TGC TGG CTG CCC TAC AAC CTG
Phe Ala Val Val Leu Ile Phe Leu Leu Cys Trp Leu Pro Tyr Asn Leu

GTC CTG CTG GCA GAC ACC CTC ATG AGG ACC CAG GTG ATC CAG GAG ACC
Val Leu Leu Ala Asp Thr Leu Met Arg Thr Gln Val Ile Gln Glu Thr

TGT GAG CGC CGC AAT CAC ATC GAC CGG GCT CTG GAT GCC ACC GAG ATT
Cys Glu Arg Arg Asn His Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile

CTG GGC ATC CTT CAC AGC TGC CTC AAC CCC CTC ATC TAC GCC TTC ATT
Leu Gly Ile Leu His Ser Cys Leu Asn Pro Leu Ile Tyr Ala Phe Ile

GGC CAG AAG TTT CGC CAT GGA CTC CTC AAG ATT CTA GCT ATA CAT GGC
Gly Gln Lys Phe Arg His Gly Leu Leu Lys Ile Leu Ala Ile His Gly

TTG ATC AGC AAG GAC TCC CTG CCC AAA GAC AGC AGG CCT TCC TTT GTT
Leu Ile Ser Lys Asp Ser Leu Pro Lys Asp Ser Arg Pro Ser Phe Val

GGC TCT TCT TCA GGG CAC ACT TCC ACT ACT CTC TAAGACCTCC TGCC
Gly Ser Ser Ser Gly His Thr Ser Thr Thr Leu
```

FIG. 2
(PAGE 2 OF 2)

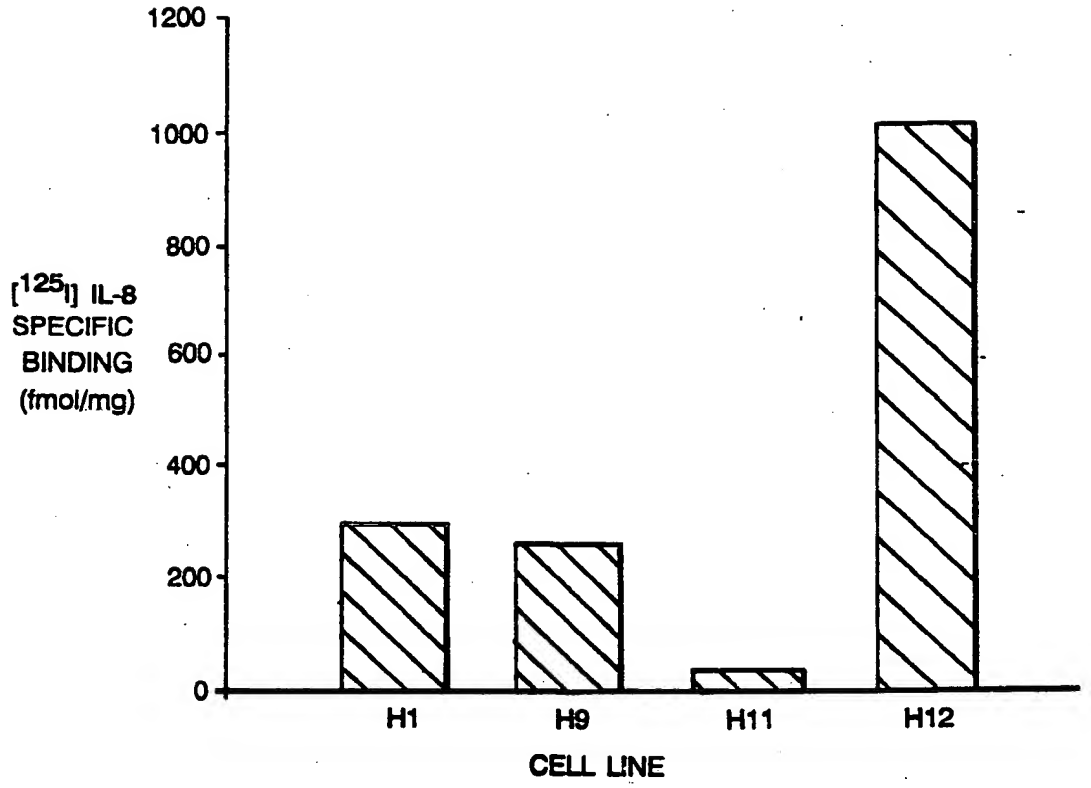
1200
1000
800
600
400
200
0
[125I] IL-8 SPECIFIC BINDING (fmol/mg)
H1
H9
H11
H12
CELL LINE

FIG. 3

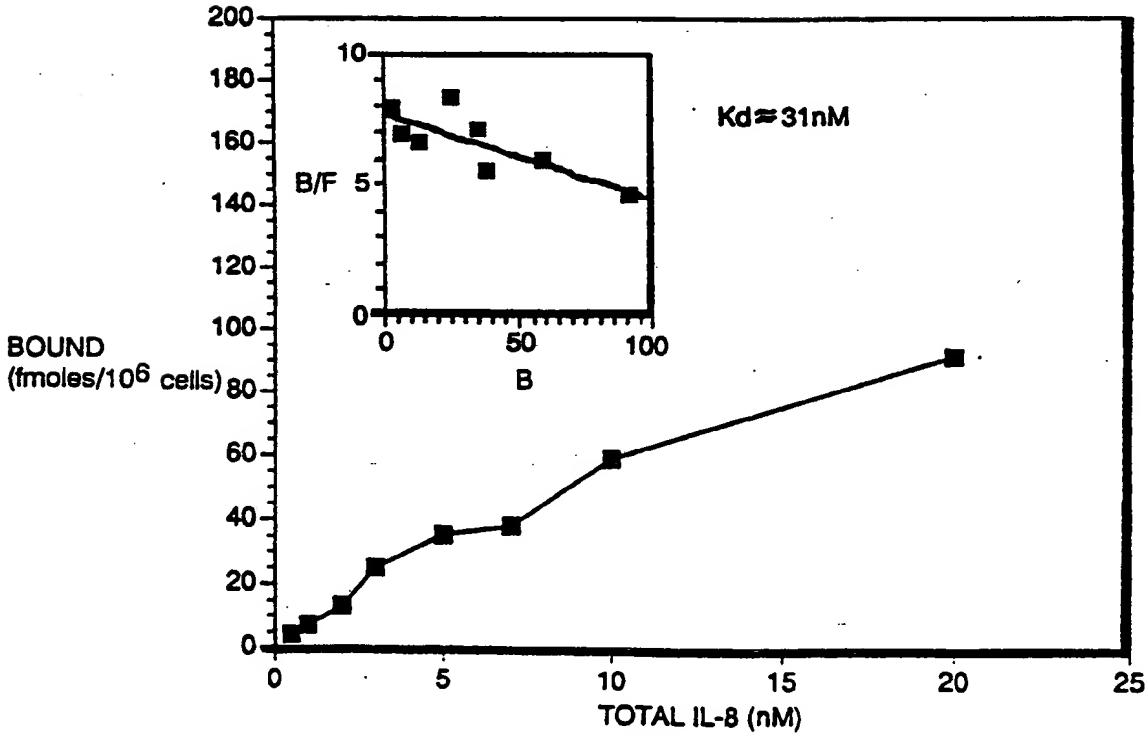
200
180
160
140
120
100
80
60
40
20
0
BOUND (fmoles/10^6 cells)
10
B/F 5
0
0
50
100
B
Kd≈31nM
0
5
10
15
20
25
TOTAL IL-8 (nM)

FIG. 4

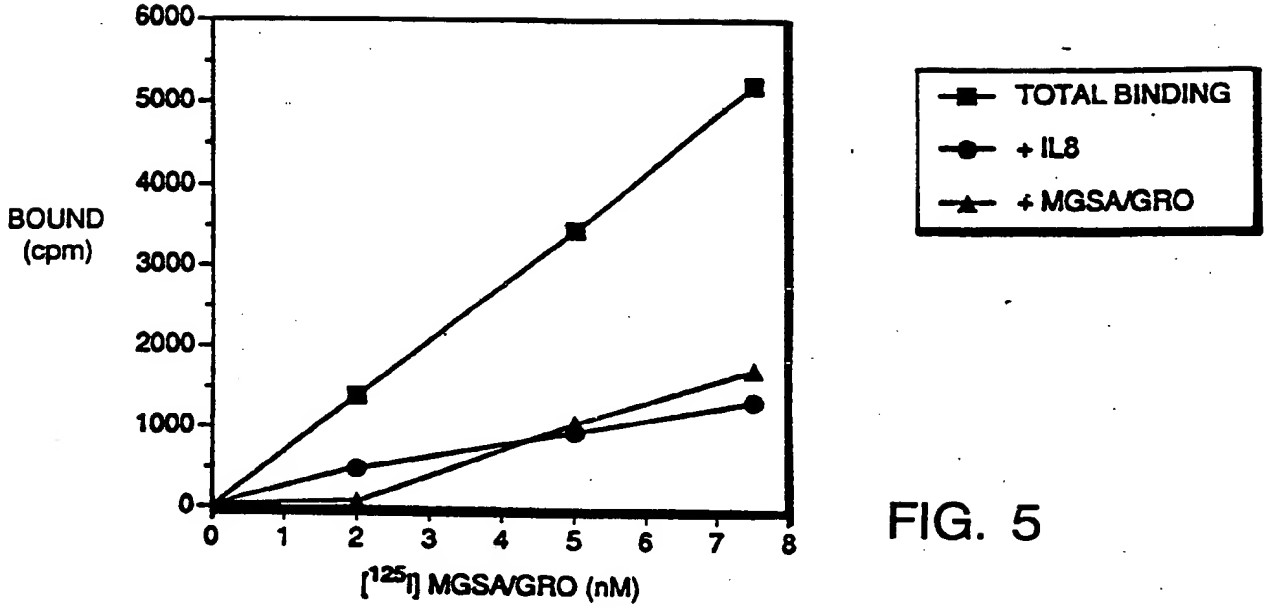
6000
5000
4000
3000
2000
1000
0
BOUND
(cpm)
0 1 2 3 4 5 6 7 8
[125I] MGSA/GRO (nM)
TOTAL BINDING
+ IL8
+ MGSA/GRO

FIG. 5

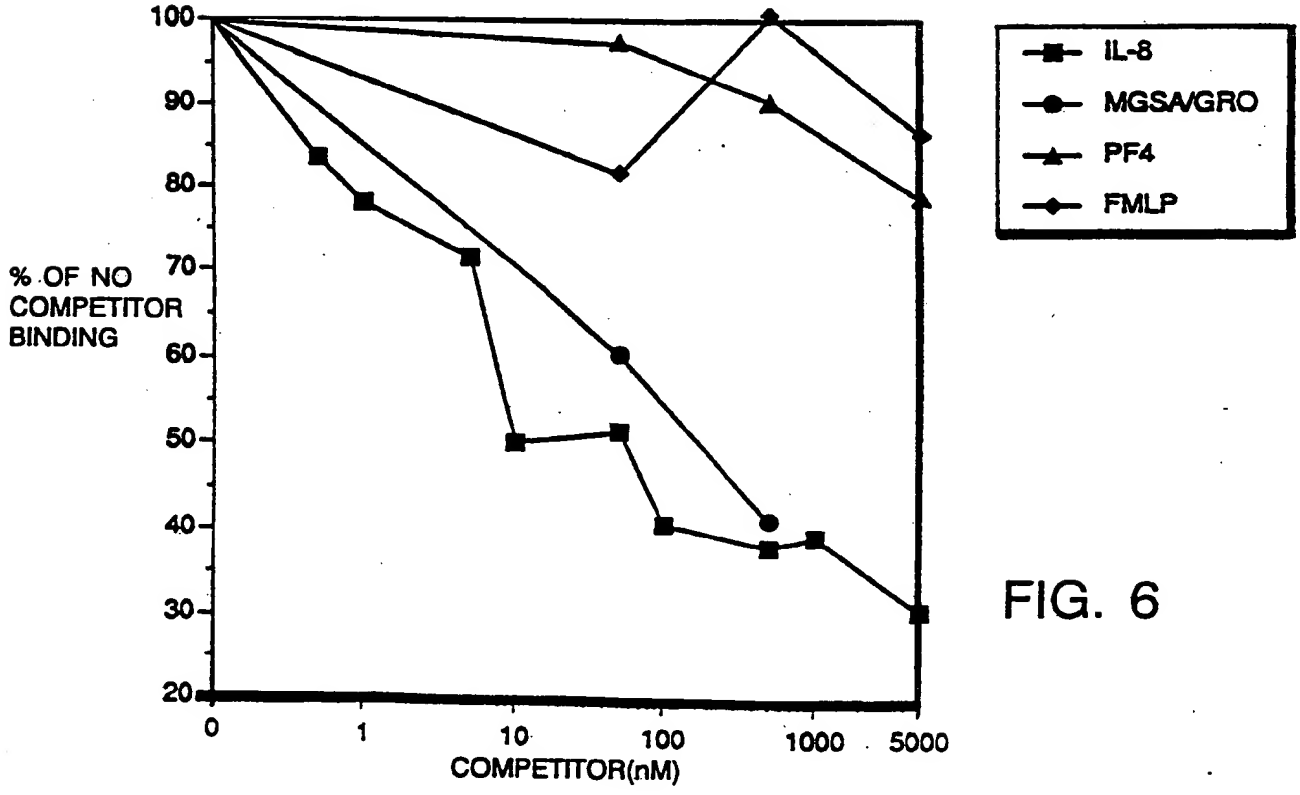
100
90
80
70
60
50
40
30
20
% OF NO
COMPETITOR
BINDING
0 1 10 100 1000 5000
COMPETITOR(nM)
IL-8
MGSA/GRO
PF4
FMLP

FIG. 6

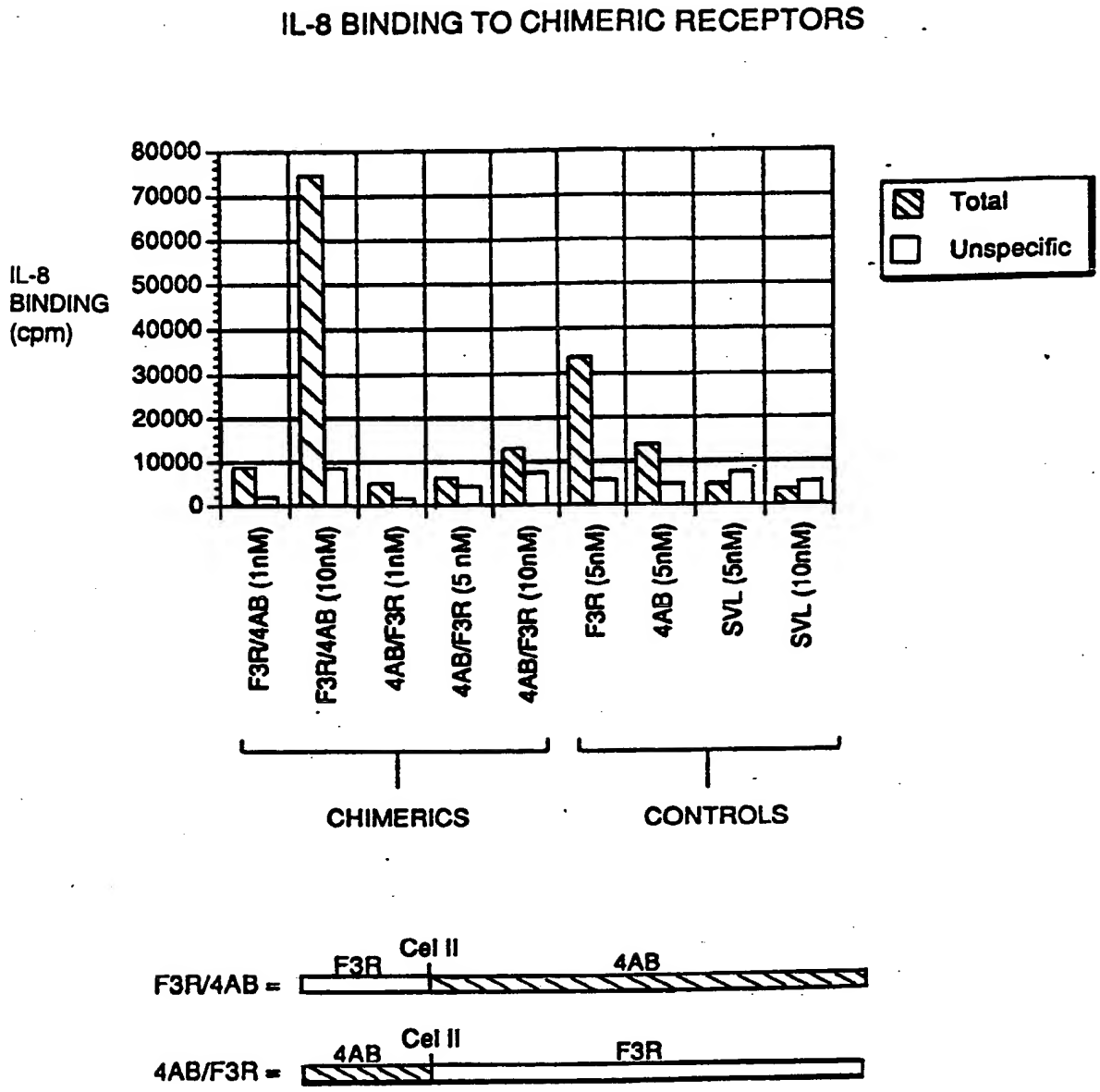
IL-8 BINDING TO CHIMERIC RECEPTORS
IL-8 BINDING (cpm)
80000
70000
60000
50000
40000
30000
20000
10000
0
Total
Unspecific
F3R/4AB (1nM)
F3R/4AB (10nM)
4AB/F3R (1nM)
4AB/F3R (5 nM)
4AB/F3R (10nM)
F3R (5nM)
4AB (5nM)
SVL (5nM)
SVL (10nM)
CHIMERICS
CONTROLS
F3R/4AB =
F3R
Cel II
4AB
4AB/F3R =
4AB
Cel II
F3R

FIG. 7

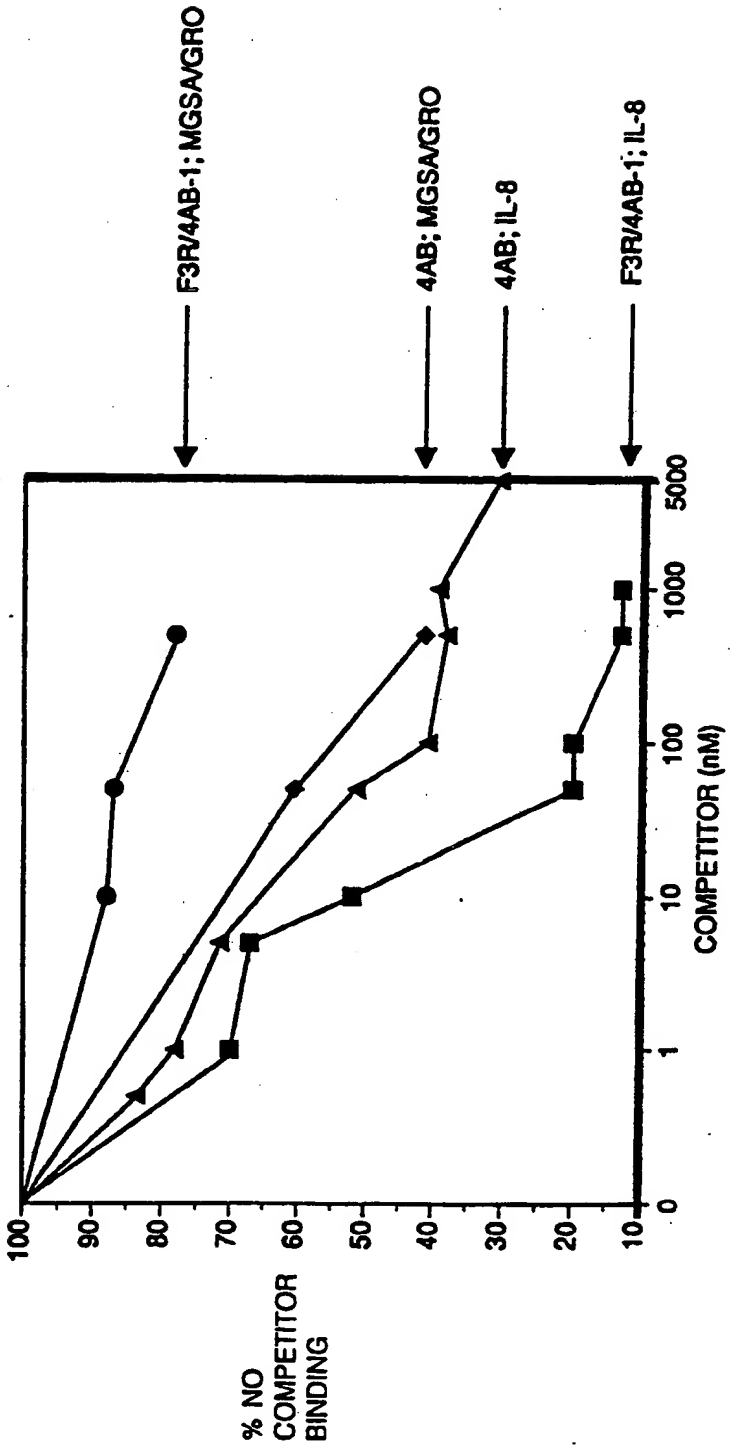
F3R/4AB-1; MGSA/GRO
4AB; MGSA/GRO
4AB; IL-8
F3R/4AB-1; IL-8
% NO COMPETITOR BINDING
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
5000
COMPETITOR (nM)
F3R/4AB-1
F3R
Cell II
4AB

FIG. 8

```
GGGAATTCCG CGAGCCCGCT CACAGGCAGT GGCTGTCGCA GCAACAGCAC GATTTAAGAC  60

TATCTCAGAA  ATG CAA GAG TTT ACC TGG GAG AAT TAC AGC TAT GAA GAT    109
            Met Gln Glu Phe Thr Trp Glu Asn Tyr Ser Tyr Glu Asp
             1               5                  10

TTT TTC GGC GAT TTC AGC AAT TAG AGT TAC AGC AGT GAC CTA CCC CCT    157
Phe Phe Gly Asp Phe Ser Asn Tyr Ser Tyr Ser Thr Asp Leu Pro Pro
    15                  20                  25

ACC CTG CTA GAC TCT GCT CCG TGC CGG TCA GAA TCT CTG GAA ACC AAC    205
Thr Leu Leu Asp Ser Ala Pro Cys Arg Ser Glu Ser Leu Glu Thr Asn
30                  35                  40                  45

AGC TAT GTT GTG CTC ATC ACC TAT ATC GTG GTC TTC CTG CTG AGC CTG    253
Ser Tyr Val Val Leu Ile Thr Tyr Ile Leu Val Phe Leu Leu Ser Leu
                50                  55                  60

CTG GGC AAC TCC CTG GTG ATG CTG GTC ATC CTG TAC AGC CGG AGC ACC    301
Leu Gly Asn Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg Ser Thr
            65                  70                  75

TGC TGG GTC ACC GAC GTC TAC CTC CTG AAC CTG GCC ATC GCC GAC CTG    349
Cys Ser Val Thr Asp Val Tyr Leu Leu Asn Leu Ala Ile Ala Asp Leu
        80                  85                  90

CTC TTT GCC AGC ACC TTG GCC ATC TGG GCC GCC TCC AAG GTG CAC GGC    397
Leu Phe Ala Thr Thr Leu Pro Ile Trp Ala Ala Ser Lys Val His Gly
    95                  100                 105

TGG ACT TTC GGC ACG GCC CTG TGT AAG GTG GTC TCG CTT GTG AAG GAA    445
Trp Thr Phe Gly Thr Pro Leu Cys Lys Val Val Ser Leu Val Lys Glu
110                 115                 120                 125

GTC AAC TTC TAC AGC GGA ATC CTG CTC CTG GCC TGC ATC AGT GTG GAC    493
Val Asn Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp
                130                 135                 140

CGC TAC CTG GCC ATC GTG CAT GCC AGA CGC ACC ATG ATC CAG AAG CGC    541
Arg Tyr Leu Ala Ile Val His Ala Thr Arg Thr Met Ile Gln Lys Arg
            145                 150                 155

CAC TTG GTC AAG TTC ATA TGC TTA AGC ATG TGG GGA GTG TCT TTG ATC    589
His Leu Val Lys Phe Ile Cys Leu Ser Met Trp Gly Val Ser Leu Ile
        160                 165                 170

CTG TCT CTG CCC ATC TTA CTG TTC CGT AAT GCC ATC TTC CCA CCC AAT    637
Leu Ser Leu Pro Ile Leu Leu Phe Arg Asn Ala Ile Phe Pro Pro Asn
    175                 180                 185

CCC AGC CCG GTC TGC TAT GAG GAC ATG GGG AAC AGC ACT GCG AAA TGG    685
Ser Ser Pro Val Cys Tyr Glu Asp Met Gly Asn Ser Thr Ala Lys Trp
190                 195                 200                 205

CGC ATG GTG CTG CGG ATG CTG CCT CAG ACT TTC GGC TTC ATC CTG CCG    733
Arg Met Val Leu Arg Ile Leu Pro Gln Thr Phe Gly Phe Ile Leu Pr
                210                 215                 220
```

FIG. 9

(PAGE 1 OF 2)

```
CTG CTG GTC ATG CTG TTT TGC TAT GTG TTC ACC CTG CGC ACG CTG TTC    781
Leu Leu Val Met Leu Phe Cys Tyr Val Phe Thr Leu Arg Thr Leu Phe
            225                 230                 235

CAG GCC CAC ATG GGG CAG AAG CAC CGG GCC ATG CGG GTC ATC TTC GCC    829
Gln Ala His Met Gly Gln Lys His Arg Ala Met Arg Val Ile Phe Ala
        240                 245                 250

GTC GTG CTC ATC TTC CTT CTC TGT TGG CTG GCC TAC AAC CTG GTT CTG    877
Val Val Leu Ile Phe Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu
    255                 260                 265

CTC ACA GAC ACC CTC ATG AGC ACC CAC GTG ATC CAG GAG ACG TGT GAG    925
Leu Thr Asp Thr Leu Met Arg Thr His Val Ile Gln Glu Thr Cys Glu
270                 275                 280                 285

CGC CGC AAT GAC ATT GAC CGG GCC CTG GAG GCC ACC GAG ATT CTG GGC    973
Arg Arg Asn Asp Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly
                290                 295                 300

TTC CTG CAC AGC TGC CTC AAC CCC ATC ATC TAC GCC TTC ATT GGC CAA    1021
Phe Leu His Ser Cys Leu Asn Pro Ile Ile Tyr Ala Phe Ile Gly Gln
            305                 310                 315

AAG TTT CGC TAT GGC CTG CTC AAG ATC CTG GCG GCC CAC GGC CTG ATC    1069
Lys Phe Arg Tyr Gly Leu Leu Lys Ile Leu Ala Ala His Gly Leu Ile
        320                 325                 330

AGC AAG GAG TTC CTG GCC AAG GAG AGC AGG CCT TCC TTT GTC GCC TCC    1117
Ser Lys Glu Phe Leu Ala Lys Glu Ser Arg Pro Ser Phe Val Ala Ser
    335                 340                 345

TCT TCA GGG AAC ACC TCT ACC ACC CTC TAA GACGCCTATG TGGGCTGCAG TCTCTCGGGC 1177
Ser Ser Gly Asn Thr Ser Thr Thr Leu End
350                 355

TTCCTCCCTC GCTTGGACAT CTCATCCCAA GNCTCATATC CTGCTCCCGG AGTCAACACA  1237

GTCCTCACTG TGGTTATAGA AAAGAGCGGN GGGCACTTCC TCAGTAGGTC CCCAGTGTAC  1297

AGNTTAGAAA GNCTGATCGG GNCCCTGTCA CTTCCCATAA TTACTCTNTC AACTAGGGGA  1357

ATCTTCTCAT TTCTAG                                                  1373
```

FIG. 9
(PAGE 2 OF 2)

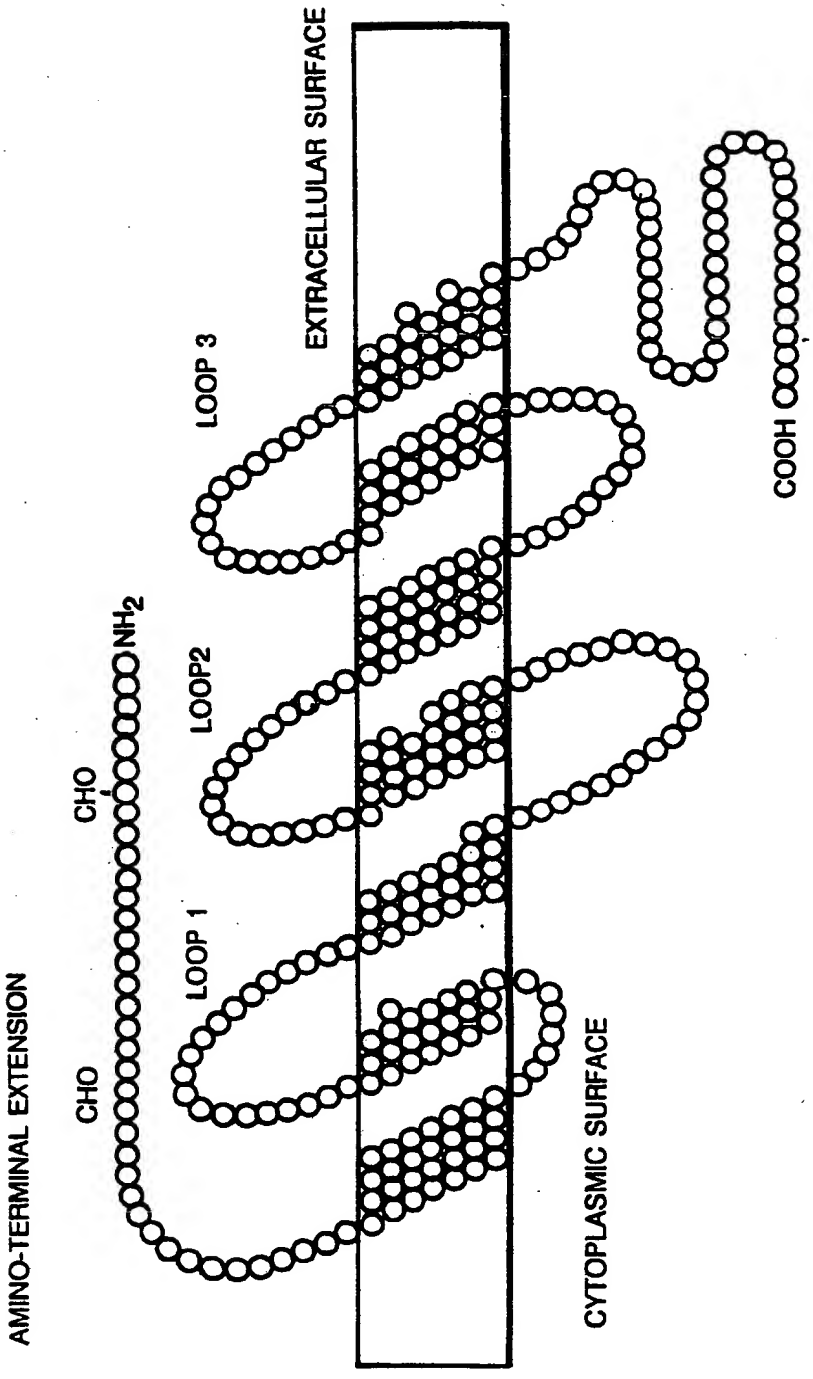
AMINO-TERMINAL EXTENSION
CHO
CHO
NH_2
LOOP 1
LOOP2
LOOP 3
EXTRACELLULAR SURFACE
CYTOPLASMIC SURFACE
COOH

FIG. 10

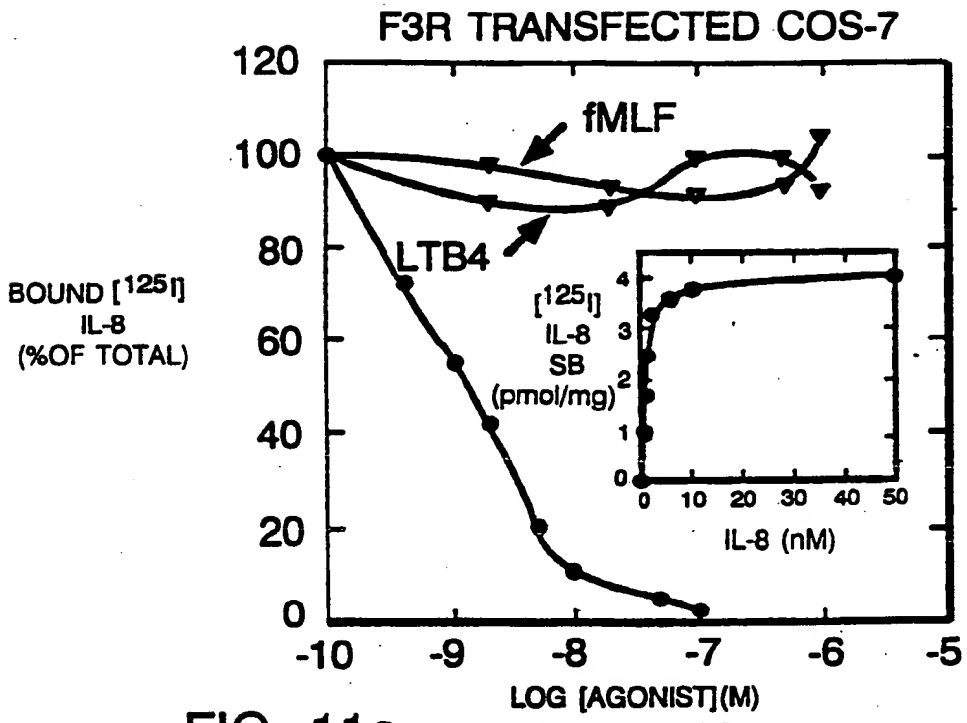

FIG. 11a Kd=1.4nM

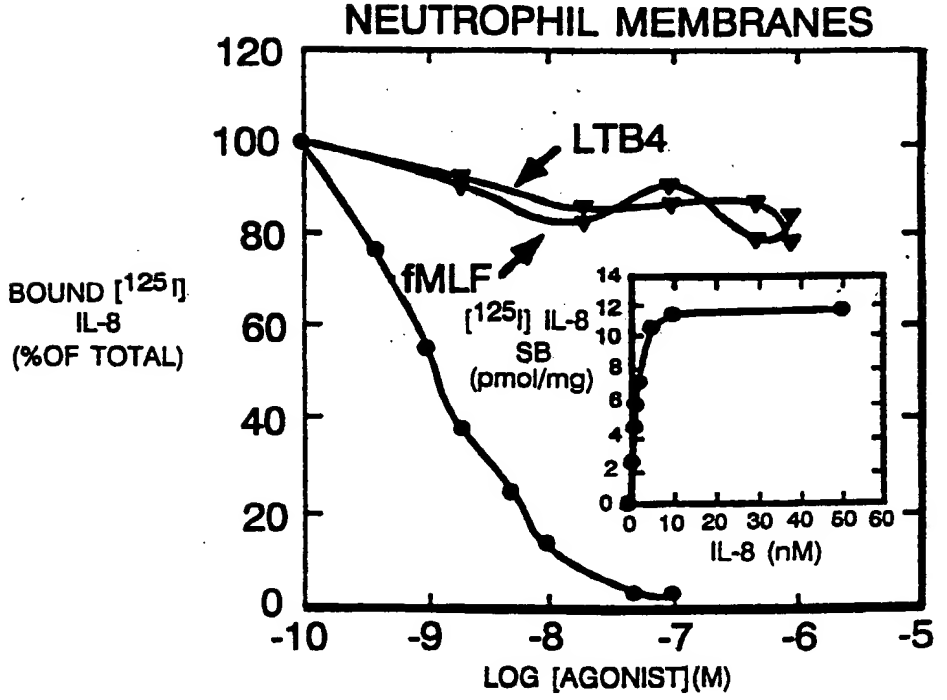

FIG. 11b Kd=1.2nM

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/02977

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12P 21/06
US CL : 435/69.1

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	435/69.1

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

APS, Dialog, Intellegenetics

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
T	J. Immunol., Vol. 148, No. 4, issued 04 February 1992, J. Lee et al., "Characterization of complementary DNA clones encoding the rabbit IL-8 receptor", pages 1261-1264, see entire document and Beckmann et al. (Fig. 1), Thomas et al.	1-17
Y,P,L	Biochem. and Biophys. Res. Commun., Vol. 179, No. 2, issued 16 September 1991, M.P. Beckmann et al., "Molecular characterization of the interleukin-8 receptor", pages 784-789, see entire document.	1-17
X,P	J. Biol. Chem., Vol. 266, No. 23, issued 15 August 1991, K.M. Thomas et al., "The interleukin-8 receptor is encoded by a neutrophil-specific cDNA clone, F3R", pages 14839-14841, see entire document.	1-17
Y,P	J. Biol. Chem., Vol. 266, No. 16, issued 05 June 1991, B. Moser et al., "Neutrophil-activating peptide 2 and gro/melanoma growth-stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils", pages 10666-10671, see entire document.	1-17

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
18 June 1992	26 JUN 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	KAREN COCHRANE CARLSON, PH.D.

Form PCT/ISA/210 (second sheet)(May 1986) 8

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	J. Biol. Chem., Vol. 265, N . 1, issued 05 January 1990, A.K. Samanta et al., "Int rleukin 8 (monocyt - derived neutrophil chemotactic factor) dynamically regulates its own receptor expression on human neutrophils", pages 183-189, see entire document.	1-17
Y	J. Biol. Chem., Vol. 265, No. 14, issued 15 May 1990, P.M. Grob et al., "Characterization of a receptor for human monocyte-derived neutrophil chemotactic factor/interleukin-8", pages 8311-8316, see entire document.	1-17

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No.[18]
Y	J. Exp. Med., Vol. 169, issued March 1989, A.K. Samanta et al., "Identification and characterization of specific receptors for monocyte-derived neutrophil chemotactic factor (MDNCF) on human neutrophils", pages 1185-1189, see entire document.	1-17